Vol. XLVI No. 6

June 1957

SCIENTIFIC EDITION

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

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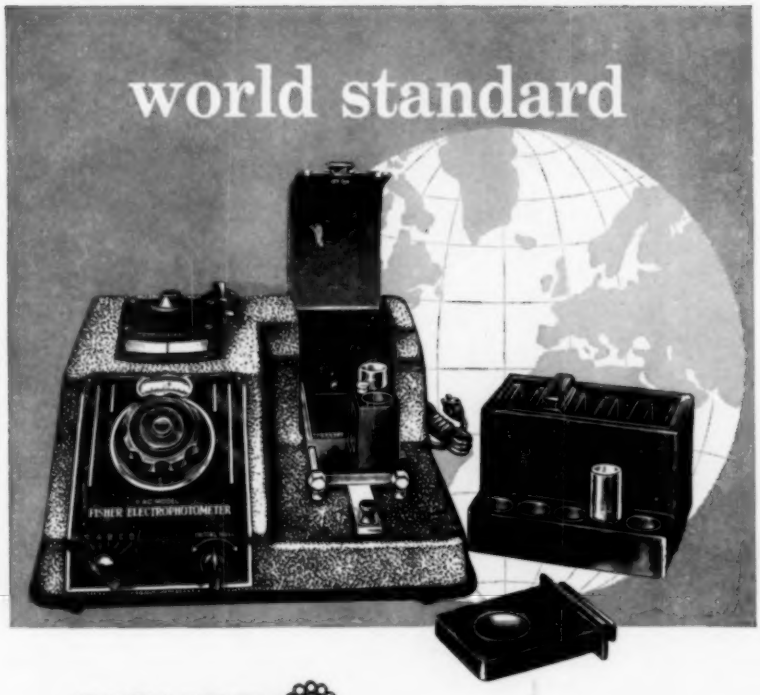

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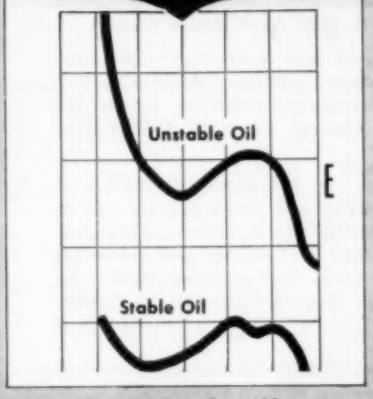

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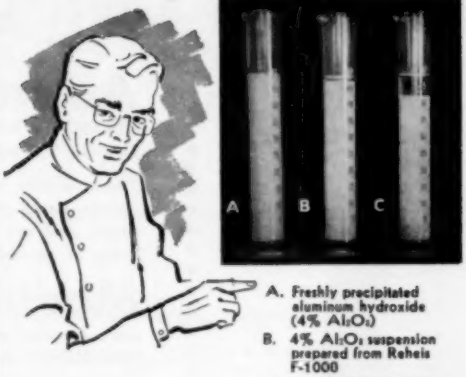

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CONSECUTIVE NO. 12

Piperidines with Motor Depressor and Anti-Inflammatory Properties*

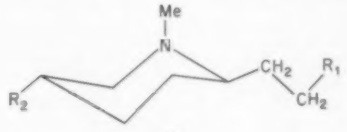
By SEYMOUR L. SHAPIRO, HAROLD SOLOWAY, and LOUIS FREEDMAN

Analogs of chlorpromazine were studied in which the amino moiety was rendered less flexible by incorporation as part of a piperidine ring system. The results indicate that this structural factor is associated with retention of depressor effects on motor activity, and also introduces anti-inflammatory properties as a pharmacological characteristic of the compounds studied.

AN INVESTIGATION of the concept of a "specific receptor surface" (1) for the ataraxic drugs (2) was undertaken to establish the degree of structural variation permissive with retention of the broad pharmacologic pattern of activity observed with 10(γ-dimethylaminopropyl)-2-chlorophenothiazine (chlorpromazine).

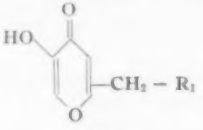
In this study the heteroaromatic moiety was varied to include phenothiazine (3) and carbazole (4) in place of the 2-chlorophenothiazine portion of chlorpromazine, and in place of the dimethylaminopropyl moiety, the less flexible (4, 5, 6) 2-(1-methyl-2-piperidyl)ethyl and 2-(5-ethyl-1-methyl-2-piperidyl)ethyl side chains were introduced.

The analogs prepared are represented by I and the corresponding methyl quaternary salts (QI).



(I)

Ia R_1 = 10-phenothiazine; R_2 = H
Ib R_1 = 10-phenothiazine; R_2 = C_2H_5
Ic R_1 = 9-carbazole; R_2 = H
Id R_1 = 9-carbazole; R_2 = C_2H_5



IIa R_1 = 10-phenothiazine
IIb R_1 = 9-carbazole

(II)

An additional variant represented by II has the polar kojyl group substituted for the dialkylaminoalkyl portion of the molecule.

Synthesis of the compounds proceeded readily by conventional methods. Phenothiazine or carbazole was condensed with a halide of the type, 2-(2-chloroethyl)-1-methylpiperidine in the presence of a basic condensing agent such as sodamide.

The pharmacological findings are summarized in Table I. The depression in motor activity was measured using rats in activity cages (7, 8) and

* Received February 14, 1957, from the Research Laboratories, U. S. Vitamin Corporation, 26 Vark Street, Yonkers, N. Y.

The authors are grateful to Dr. G. Ungar and his staff for the pharmacological evaluation of the compounds herein described.

has been expressed as per cent reduction in motor activity (RMA), and the anti-inflammatory effect measured (9, 10) at 50 per cent inhibition of the response expressed as units per gram of tested drug.

TABLE I.—PHARMACOLOGICAL RESULTS WITH TEST COMPOUNDS[a]

Code No.	LD min.[c]	RMA[b] Test Level, mg./Kg.	RMA[b] Red., %	AI[c] Units/Gm.
Ia	100	20	40	35
QIa[d]	100	20	73	50
Ib	200	..	..	13
QIb	50	20	28	60
IIa	50	10	Neg.	32
Ic	250	50	33	Neg.
QIc	50	15	15	30
Id	100	20	47	20
QId	70	15	53	50
IIb	450	90	Neg.	Neg.
Chlorpromazine		10	71	..
Butazolidin		..	..	12

[a] Minimum lethal dose was established subcutaneously in mice.

[b] The reduction in motor activity (RMA) is performed in rats. The drug is administered subcutaneously and the rat placed in a cage wired with a counter which is responsive to movements of the rat. The counter records and sums up the extent of spontaneous activity. The duration of the experiment is 18 hours. Each rat acts as his own control by being given a drug one day and saline the next day. At least six rats are used at each dose level tested. Activity of a drug is reported as average % change (% red.) from the control value.

[c] Anti-inflammatory response (AI) was evaluated by administration of the compounds subcutaneously.

[d] Tested as methobromide; all other quaternaries tested as methiodides.

[e] Other pharmacological findings of interest established by intravenous injection at dosage levels corresponding to 1/100 of LDmin. in the anesthetized dog. Antihistamine activity is shown by Ia, QIa, and QIb; marked hypotensive action by QIa, QIb, QIc, and QId.

The activity measurements reflect that all of the I compounds induce some depression in motor activity with the best compound being QIa. Within the I series, R_2 = ethyl showed better response than R_2 = hydrogen in the carbazole series, and the reverse effect was noted in the phenothiazine compounds.

The type II structures did not show reduction in motor activity. Significant anti-inflammatory activity was obtained, particularly with the quaternary structures QIa, QIb, and QId.

It is of interest that in this work the quaternary compounds are generally more active than the tertiary amines, in contrast to the findings with chlorpromazine and promethazine (11).

EXPERIMENTAL[1,2]

5-Ethyl-2-piperidine-ethanol.—A solution of 30.2 Gm. (0.2 mole) of 5-ethyl-2-pyridine-ethanol in 22 ml. of glacial acetic acid containing 1.0 Gm. of platinum oxide was shaken in the hydrogenator (Parr) at room temperature at 50–60 lb. of hydrogen pressure. After twenty-four hours (50% of the theoretical hydrogen uptake), an addtional 1 Gm. of catalyst was added and after twenty-nine hours, 98% of theoretical hydrogen was taken up. The catalyst was separated and the reaction mixture concentrated to 50 ml. under vacuum, treated with excess 40% sodium hydroxide solution, and the product extracted with 500 ml. of ether. The ethereal extract dried over anhydrous magnesium sulfate was then filtered. The ether was removed and the product distilled, there being obtained 21.3 Gm. (68%) of water-white liquid, b. p. 168–170°/50 mm.

Treatment with ethereal hydrogen chloride yielded the hydrochloride which was recrystallized (isopropyl alcohol-isopropyl ether) to give a 72% recovery of product, m. p. 125–127.°

Anal. Calcd. for $C_9H_{20}ClNO$: C, 55.8; H, 10.4. Found: C, 56.3; H, 10.3.

5-Ethyl-1-methyl-2-piperidine-ethanol.—A solution of 34.6 Gm. (0.22 mole) of 5-ethyl-2-piperidine-ethanol in 25 ml. of acetic acid and 19.5 ml. (0.26 mole) of 37% formaldehyde was treated with palladium on charcoal (prepared by dissolving 0.25 Gm. of palladium chloride in 10 ml. of water containing 0.1 ml. of 3*N* hydrochloric acid and 2.5 Gm. of charcoal (Darco), and diluted with 60 ml. of water).

Upon hydrogenation for twenty-five hours at 30–50° under 50–60 lb., 93% of the theoretical amount of hydrogen had been taken up. After removal of the catalyst, the reaction mixture was treated with excess 40% sodium hydroxide. The oil which separated out and the aqueous alkaline phase were extracted with 500 ml. of ether, and the ether extract dried over anhydrous magnesium sulfate. The ethereal extract was filtered, the ether removed, and the product distilled, there being obtained 29.8 Gm. (79%) of 5-ethyl-1-methyl-2-piperidine-ethanol, b. p. 114–116°/4–7 mm.

Anal. Calcd. for $C_{10}H_{21}NO$: C, 70.1; H, 12.4; N, 8.2. Found: C, 70.2; H, 12.6; N, 7.8.

2-(2-Chloroethyl)-5-ethyl-1-methylpiperidine Hydrochloride.—A solution of 24.1 Gm. (0.14 mole) of 5-ethyl-1-methyl-2-piperidine-ethanol in 100 ml. of chloroform was cooled in an ice bath and a stream of dry hydrogen chloride in excess passed through. The ice bath was removed and 35.6 Gm. (0.3 mole) of thionyl chloride added dropwise at a rate sufficient to maintain reflux. After addition of thionyl chloride had been completed, external heating was applied and the mixture refluxed one hour. The reaction mixture was cooled, 30 ml. of ethanol added and about 100 ml. of solvent distilled out; 100 ml. of benzene was added and the reaction mixture concentrated to a reddish syrup by removal of low boiling components. On standing, the product crystallized and after trituration with ether afforded 29.8 Gm. (94%) of white solid, melting at 65–69°.

Anal. Calcd. for $C_{10}H_{21}Cl_2N$: C, 53.1; H, 9.4; Cl, 31.4. Found: C, 53.0; H, 9.4; Cl, 30.7.

10-[2-(5-Ethyl-1-methyl-2-piperidinyl)ethyl]phenothiazine (Ib).—A suspension of 4.3 Gm. (0.11 mole) of sodamide in 100 ml. of toluene in an anhydrous system was treated with 19.9 Gm. (0.1 mole) of phenothiazine and refluxed for two and one-half hours. The reaction mixture was cooled and 60 ml. of a toluene solution of 2-(2-chloroethyl)-5-ethyl-1-methylpiperidine (prepared by treating 27.2 Gm. (0.12 mole) of an aqueous solution of the hydro-

[1] Melting points are not corrected.

[2] Analyses by Weiler and Strauss, Oxford, England.

chloride with 40% sodium hydroxide, extracting the free base with toluene, and drying the toluene extract over anhydrous magnesium sulfate and filtering) was added dropwise. Upon completion of addition external heat was applied and the reaction mixture refluxed twenty hours. The reaction mixture was cooled, the separated sodium chloride filtered off, and the filtrate extracted with 3 *N* hydrochloric acid. The acid layer was separated, and made alkaline with 40% sodium hydroxide, and the separated oil extracted with ether. The ether extract was treated with charcoal, filtered, dried over anhydrous magnesium sulfate and treated with 50 ml. of 8% ethereal hydrogen chloride. There was thus obtained the hydrochloride of the product 8 Gm. (21%) which, after drying and recrystallization from methyl ethyl ketone, melted at 171–173°.

Anal. Calcd. for $C_{22}H_{29}ClN_2S$: C, 67.9; H, 7.5; N, 7.2. Found: C, 68.0; H, 7.8; N, 7.3.

The methiodide of the above compound was prepared by treating an acetonitrile solution of its free base with excess methyl iodide, allowing it to stand overnight, and then adding ether to excess. The ether-insoluble product, a white water-soluble powder, melted at 106–109°.

Anal. Calcd. for $C_{23}H_{31}IN_2S$: N, 6.1. Found: N, 5.7.

The methobromide (QIb) was prepared by treating an ethereal solution of the free base with excess methyl bromide. The reaction mixture was stoppered and allowed to stand at 5° for forty-eight hours. The precipitate of product so obtained, recrystallized from acetonitrile-isopropyl ether, melted at 187–189°.

Anal. Calcd. for $C_{23}H_{31}BrN_2S$: C, 61.7; H, 7.0; N, 6.3. Found: C, 61.4; H, 7.2.; N, 6.2.

9-[2-(5-Ethyl-1-methyl-2-piperidinyl)ethyl]carbazole (Id).—A suspension of 3.9 Gm. (0.1 mole) of sodamide in 125 ml. of xylene in an anhydrous system was treated with 16.7 Gm. (0.1 mole) of carbazole and refluxed for one and one-half hours. The reaction mixture was cooled and treated dropwise for over an hour with a xylene solution of 2-(2-chloroethyl)-5-ethyl-1-methylpiperidine (prepared from 24.9 Gm. (0.11 mole) of the hydrochloride). After addition was complete the reaction mixture was refluxed for two hours and then allowed to stand overnight. The reaction mixture was filtered and processed at this point analogous to Ib.

There was thus obtained the hydrochloride of the product which, recrystallized from methyl ethyl ketone, afforded 5.6 Gm. (16%) melting at 144–147°.

Anal. Calcd. for $C_{22}H_{29}ClN_2$: Cl, 9.9; N, 7.9. Found: Cl, 10.0; N, 7.9.

The methiodide (QId) of the free base of the above compound was prepared by treating an acetonitrile solution of its free base with excess methyl iodide, allowing to stand overnight and removing the volatile products under vacuum to leave a residue of the product, which after trituration with ether and recrystallization from hexane-methyl ethyl ketone melted at 92–95°.

Anal. Calcd. for $C_{23}H_{31}IN_2$: N, 6.1. Found: N, 5.7.

The required 2-(2-chloroethyl)-1-methylpiperidine is preparable from the commercially available 2-pyridine-ethanol by the sequence of reactions indicated above. Alternatively, it is prepared as follows.

2-(2-Hydroxyethyl)-1-methylpyridinium Bromide.—A solution of 37 Gm. (0.3 mole) of 2-pyridine-ethanol and 57 Gm. (0.6 mole) of methyl bromide in 200 ml. of acetonitrile was maintained overnight and then the volatiles removed under vacuum distillation. The product remained as an almost colorless oil and was used for the next step without further purification.

(1-Methyl-2-piperidine)ethanol.—All of the methobromide above was dissolved in 250 ml. of methanol and 0.5 Gm. of platinum oxide added, and then the reaction mixture was hydrogenated at room temperature with shaking at 60 lb. hydrogen pressure for seven hours at which point 80% of the theoretical amount of hydrogen had been taken up. The reaction mixture was filtered from the catalyst, the methanol removed from the filtrate under vacuum, and the hydrobromide of the product crystallized. The hygroscopic hydrobromide was dissolved in 300 ml. of water, made alkaline with 40% sodium hydroxide, the solution saturated with potassium carbonate, and extracted successively with five 75-ml. portions of ether. The ethereal extracts were combined, dried over anhydrous magnesium sulfate, filtered, the ether removed and the product, obtained in 73% yield distilled, b. p. 65–68°/0.15–0.25 mm.

2-(2-Chloroethyl)-1-methylpiperidine Hydrochloride.—This was prepared from (1-methyl-2-piperidine)ethanol and thionyl chloride as described above. The product so obtained in 83% yield melted at 135–136°.

Anal. Calcd. for $C_8H_{17}Cl_2N$: C, 48.5; H, 8.7; N, 7.1. Found: C, 48.0; H, 8.6; N, 6.8.

10-[2-(1-Methyl-2-piperidinyl)ethyl]phenothiazine (Ia).—A suspension of 3.91 Gm. (0.1 mole) of sodamide in 125 ml. of xylene in an anhydrous system was treated with 19.9 Gm. (0.1 mole) of phenothiazine and refluxed for two hours. The reaction mixture was maintained at reflux and a solution of 2-(2-chloroethyl)-1-methylpiperidine in 90 ml. of xylene (prepared from 21.8 Gm. (0.11 mole) of the hydrochloride) was added dropwise over one and one-half hours. Refluxing and stirring was continued for an additional two and one-half hours and then the reaction mixture was allowed to stand overnight. The precipitated sodium chloride was filtered off and the filtrate extracted with 250 ml. of water containing 0.3 mole of hydrogen chloride. The aqueous phase was made basic with 40% sodium hydroxide and extracted with ether. The ethereal extracts were combined, dried over anhydrous magnesium sulfate, filtered, and the ether removed leaving 33 Gm. of dark oily residue. After standing several days, crystallization was noted and the material was washed with a minimum of pentane. The pentane-insoluble portion was recrystallized from boiling hexane and yielded 8 Gm. (28%) of the free base of the product, m. p. 90–91°.

Anal. Calcd. for $C_{20}H_{24}N_2S$: C, 74.0; H, 7.5; N, 8.6. Found: C, 73.7; H, 7.4; N, 8.9.

The methobromide (QIa) was prepared and melted at 215–217° (isopropyl alcohol-isopropyl ether).

Anal. Calcd. for $C_{21}H_{27}BrN_2S$: N, 6.7. Found: N, 7.1.

9-[2-(1-Methyl-2-piperidinyl)ethyl]carbazole (Ic).—To a cooled, stirred solution of 1.9 Gm. (0.055 mole) of sodamide in 300 ml. of anhydrous liquid

ammonia was added 8.4 Gm. (0.05 mole) of carbazole.

After the carbazole had been added the cooling bath was removed and after one hour, 200 ml. of dry toluene was added slowly, and the ammonia allowed to distil out. After two hours external heat was applied and the reaction mixture refluxed for two hours to remove the remainder of the ammonia. The reaction mixture was cooled and a solution of 2-(2-chloroethyl)-1-methylpiperidine in 60 ml. of toluene (prepared from 9.9 Gm. of the hydrochloride as previously described) was added dropwise over one-half hour. The reaction mixture was then refluxed for eight hours, cooled, and filtered from the precipitated sodium chloride. The toluene filtrate was extracted with 200 ml. of water containing 30 ml. of 3 *N* hydrochloric acid, the aqueous layer separated, made strongly basic with 40% sodium hydroxide, and extracted with ether. The ether extracts were dried over anhydrous magnesium sulfate, filtered, the ether removed, and the residue rinsed with a small amount of pentane. The pentane-insoluble portion of the free base of the product, recrystallized from hexane, yielded 6.2 Gm. (42%) and melted at 120–122°.

Anal. Calcd. for $C_{20}H_{24}N_2$: C, 82.1; H, 8.3; N, 9.6. Found: C, 81.9; H, 8.2; N, 9.9.

The methiodide (QIc) of this base was prepared and melted at 208–209° (ethanol).

Anal. Calcd. for $C_{21}H_{27}IN_2$: N, 6.5. Found: 6.3.

5-Hydroxy-2-(9-carbazolylmethyl)-4H-pyran-4-one (IIb).—To 250 ml. of anhydrous ammonia was added 5.1 Gm. (0.13 mole) of sodamide (acetone-dry ice), followed by 23.7 Gm. (0.14 mole) of carbazole added portionwise over thirty minutes, and stirring and cooling continued for three hours. Kojyl chloride (12), 10.4 Gm. (0.065 mole), was then added over forty-five minutes with continued stirring and cooling for fifteen hours. The ammonia was evaporated and the residue treated with 1500 ml. of water and centrifuged to clarify. The aqueous phase was separated and acidified with concentrated hydrochloric acid and extracted successively with four 250-ml. portions of chloroform. The chloroform extracts were combined, dried over anhydrous magnesium sulfate, filtered, the chloroform evaporated and the residue of 7 Gm. recrystallized from acetonitrile. There was obtained 1.6 Gm. (8.5%) of product melting at 241–243°.

Anal. Calcd. for $C_{18}H_{13}NO_3$: C, 74.5; H, 4.2; N, 4.8. Found: C, 74.5; H, 4.4; N, 4.9.

5-Hydroxy-2-(10-phenothiazinylmethyl)-4H-pyran-4-one (IIa).—Prepared as for IIb above, there being obtained a 24% yield, m. p. 200–203° (ethanol).

Anal. Calcd. for $C_{18}H_{13}NO_3S$: C, 66.9; H, 4.1; N, 4.3. Found: C, 66.7; H, 4.3; N, 4.0.

SUMMARY

1. Synthesis and pharmacological evaluation of compounds having the 2-(1-methyl-2-piperidyl)ethyl and 2-(5-ethyl-1-methyl-2-piperidyl)-ethyl chain attached to the 10 and 9 positions of phenothiazine and carbazole, respectively, indicate that such structures, particularly as to their methyl quaternary salts, have marked anti-inflammatory action and produce reduction in motor activity in laboratory animals.

2. A polar functional group containing oxygen rather than an amino group, such as the kojyl radical, similarly attached to phenothiazine and carbazole, did not produce reduction in motor activity.

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β-(6-Methoxy-2-naphthyl)-α,α′-dimethyl-β-ethyl-propionic acid
Eriodictyol
Isorhamnetin
Epicatechin
2-Keto-L-gluonic acid

The Diphasic Actions of Ceanothyn and EDTA as Blood Coagulants*

By JACK G. BISHOP† and ALFRED W. RICHARDSON‡

Ceanothyn in specific concentrations in blood acts as a blood coagulant, and in specific larger concentrations acts as an anticoagulant. In this regard, it closely follows the similar diphasic coagulant-anticoagulant action of EDTA, a recognized calcium chelator. The coagulant action on blood as a result of the chelation of small amounts of blood calcium may have significance in the blood clotting mechanism as well as practical importance in blood storage methods.

As DESCRIBED by Taylor (1) hydroalcoholic extract of *Ceanothyn americanus* has been employed as a coagulant under the name Ceanothyn.® Groot (2) reported an average decrease of −30% in blood clotting time in response to dosages of 1/4 to 1/2 ounce of Ceanothyn preparation, and a decrease of −41.8% in blood clotting time in one group of 13 patients. He reported that blood coagulation time depression corresponded, within limits, to the amount of drug orally administered. Tharaldesen and Krawetz (3) confirmed these findings and reported that this action may occur independently of the action of digestive enzymes. Payne (4) cited 225 cases in which he used the drug preoperatively to hasten coagulation and thereby control hemorrhage. He found a decrease of −21.9% in the blood coagulation time. Some workers have found less effective results.

No one among the investigators has presented supportive evidence to clarify the mode of action of this botanical root bark extract which the more careful clinical studies seem to have indicated effectual as a blood coagulant, though variably reported in this respect.

After a review of the total situation, it was considered that it might be of interest to assay quantitatively the effectiveness of Ceanothyn as a coagulant and attempt to determine its action in the blood coagulation mechanism if it were found effective. It is believed that our findings have demonstrated Ceanothyn to be an effective coagulant, that we may have disclosed at least one reason for the variable results previously reported. Also, the studies may somewhat delineate the role of calcium ion in the process of blood coagulation, as well as the role of Ceanothyn.

* Received December 10, 1956, from the Department of Physiology, School of Medicine, Indiana University, Bloomington, Ind.
These studies were supported by grants from Flint, Eaton & Co., Decatur, Illinois.
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PROCEDURE AND METHODS

For the measurement of blood coagulation time, the recording coagulometer of Richardson and Bishop (5) was used. This apparatus utilizes a motor attached to a gear in such a manner that an affixed small platform will rotate 45° up and down from horizontal. A full oscillation occurs every 10 seconds. The platform holds a removable container for blood as well as a mercury switch. The positioning of these two components is such that the contacts of the mercury switch are 160° of arc from two platinum-rhodium electrodes in the stopper of the blood container. Since the switch and electrodes are a part of a series circuit, the switch closes the circuit only when liquid has rolled away from the probes by gravitational action. When, however, all or part of the blood has coagulated it cannot move from the probes, so that the apparatus progressively samples the clot formation at 10-second intervals. The current flowing through the circuit represents the geometric magnitude of the blood clot, and does not register the liquid blood. Control assays of the clotting time determinations reported here showed the standard errors of the means from individual animals to be 0.3 minutes, with the use of three separate units during the assays.

All blood samples were drawn from the veins of dogs, using 5-cc. syringes and No. 22 needles, both paraffin oil coated. A total sample of 4 cc. of blood was withdrawn for each assay, with 1 cc. not used. The animals ranged in weight from 7 to 19 Kg., and were anesthetized with pentobarbital sodium 33 mg./Kg. When agents were added to blood *in vitro*, one drop of the agent was added to 1 cc. of blood, the volume of the drop being previously calibrated, and dilutions of the agents were made for quantitations.

A typical record of a control blood clotting sample is shown in Fig. 1 which demonstrates a clotting time of 5 min., 40 sec. The Esterline-Angus (5 ma.) recording covers a 7-minute period. Arrows designate the beginning of acceleration (B. A.), the point in time when a visible clot was formed (V. C.) and the area of clot stabilization (C. S.). There is an inherent error (5 sec.) with the method, either on the record or visually since the blood moves every 5 seconds. It has been found reliable, within these

limits, to accept the end of the acceleration phase as the clotting point, as is reasonably apparent from a scrutiny of the curve form. This curve form of progressive blood clotting is in agreement in most respects with that shown by Nygaard (6) using a photometric method of registration. The point of

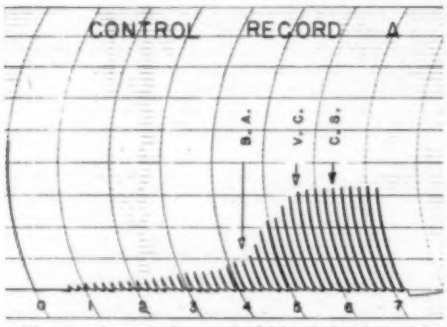

Fig. 1.—A typical record of blood clotting which demonstrates the sequence of events using the method of Richardson and Bishop. It can be seen that the slow and accelerated phases of clotting are apparent. The notation (V. C.) shows the point of visual clot, whereas (B. A.) represents beginning of acceleration, and (C. S.) clot stabilization. Lines on the ordinate are 1 minute time.

coagulation on our clotting curve has been established by over 1,900 samplings of blood, by readily observable confirmation.

The Ceanothyn used in these studies was a standard commercial pharmaceutical preparation. Further details of the blood coagulation measuring apparatus used can be found in other papers (5, 7). The solvents in Ceanothyn were assayed and were found to have no effect on blood clotting.

RESULTS

Ceanothyn Assays.—In this series, 21 animals were used to obtain 176 experimental samples and 148 control samples. Drawn blood was added to the receptacles for assay with and without various different quantities of Ceanothyn. The range of concentration in the experimental assays was 1:100,000 to 1:300 Ceanothyn-to-blood, or 10 to 3330 p. p. m., 0.001 to 0.333%. It was found that progressively increased concentrations of Ceanothyn above 1:50,000 accelerated the clotting time more and more until a concentration of 1:5000 was reached (200 p.p.m. 0.02%) at which point the clotting time had declined to 3.6 minutes, a decrease of −28% below the control (significant at 1% level of confidence) clotting time of 5.0 minutes. With further increased concentrations of Ceanothyn, clotting time was diminished less and less in a uniform manner so that with concentrations of 1:500 and greater the clotting time was significantly increased (1% level of confidence). A plot of the results may be found in Fig. 2. Whereas the results of the assays evidenced a uniform pattern, they were not easily explained within the limitations of these

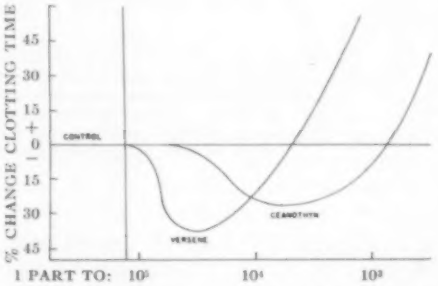

Fig. 2.—Clotting time as a function of *in vitro* concentration of Ceanothyn and EDTA (Versene) in blood. Control clotting time averaged 5.0 minutes for Ceanothyn and 5.2 minutes for Versene, total average 5.1 minutes. The diphasic clotting actions as a function of concentration is apparent.

data. The literature concerned with blood coagulation at the present date refers to only one factor in the coagulation mechanism that has shown two converse actions, with convincing confirmation. Steinberg and Brown (8), Steinberg, Segal, and Parris (9), Page, Russell, and Rosenthal (10), and Martin (11) have reported that oxalate, known to diminish blood calcium when given *in vivo* in small quantities, resulted in a decreased blood clotting time. Ceanothyn is known to contain oxalate. There was no agreement, however, that this action was on ionized calcium although Martin (11) did suggest the action to be on calcium ion. The action of large concentrations of oxalate to prolong the clotting time of blood by action on ionized calcium presumably, is well established.

In order to evaluate the effect of progressive diminution of blood calcium quantitatively on the clotting time of blood, a sequestering agent was chosen, ethylenediaminetetraacetic acid (EDTA, Versene), known to quantitatively chelate calcium.

Quantitative Additions of EDTA to Blood *in vitro*.—In one series, 11 animals were used to obtain 151 experimental samples and 96 control samples. Withdrawn blood was added as before to the blood receptacles, some with and some without various different quantities of EDTA (Versene). One cubic centimeter of the standard Versene solution chelated 32 mg. of calcium, before dilution.

In a second series, 8 animals were used to obtain 82 experimental samples and 107 control samples. The method of assay was the same, but to the experimental samples of blood were added various different quantities of disodium Versenate, weighed and diluted from dry powder stock. Chelation of calcium was calculated on a molar basis, but blood samples before and after the introduction of these two sequestering agents were assayed in the analytical chemistry laboratory for calcium in order to satisfactorily verify the calculations.

It was found that progressively increased concentrations of these two Versenates in blood resulted in comparable clotting curves with only minor deviations, so the results of the two series will be discussed as one multiple assay series. For discussion of quantities, concentration in blood, and calcium chelation, therefore, disodium Versenate has been equilibrated to the chelation of 32 mg. of calcium per cc. before dilution of the standard sequestering solution.

Delineated in Fig. 2 is a plot of the results of the Versenate studies along with the Ceanothyn studies for comparison. The range of concentration of the Versene (Versenate) standard solution in the experimental assays was 1:100,000 to 1:1100, (or 10 to 909 p.p.m.), as reported here. Stronger concentrations were added up to no clot formation, but the interpolating increments were not sufficiently spaced for more than a qualitative statement regarding critical minimal quantity for clot prevention.

It was found that progressively increased concentrations of standard Versenate above 1:100,000 accelerated blood coagulation time more and more until a concentration of 1:26,650 was reached (37.5 p.p.m. 0.00375%), a point at which the clotting time had declined to −38% below the control clotting time (significant at 1% level of confidence). With further increased concentration of standard Versenate, clotting time was diminished less and less, in a uniform manner, until with the largest concentrations, action was reversed and was prolonged. Analysis of the data shows that the maximum acceleration of clotting occurred when 1.2 μg. of calcium was chelated per cc. of blood, but that a very effective acceleration of clotting occurred when 0.7 to 4.0 μg. of calcium was chelated per cc. of blood. This effective range represents 22 to 125 p.p.m. of our standard Versenate in blood.

Having demonstrated by Versenate chelation of calcium that blood calcium depletion evidences a coagulant action or anticoagulant action, depending on the magnitude of the calcium depletion, a feasible hypothesis could be constructed to propose that Ceanothyn might display its diphasic clotting action by the depletion of calcium ion in the blood. If this were true it required about 5 times as much Ceanothyn as our standard Versenate for comparable action on calcium ion in the blood.

Less exhaustive quantitative assays were employed using sodium citrate and sodium oxalate, to determine their effect on blood coagulation. The different concentrations of each followed diphasic curves similar to those of Ceanothyn and Versenate, where small magnitudes were coagulants and large magnitudes were anticoagulants. This was confirmatory *in vitro* to the work of Steinberg and Brown (8), Steinberg, Segal, and Parris (9), Page, Russell, and Rosenthal (10), and Martin (11), but compared analytically with the other studies reported here, our work would suggest agreement with Martin that the coagulant action of citrate and oxalate in low concentration is due to action on calcium ion. Other workers have not agreed with this view, in part due to the fact that disparity had been reported between *in vivo* and *in vitro* actions.

COMMENT

Preparations of Ceanothyn have been used orally and topically as a coagulant agent. Previous work which assayed the oral effectiveness of Ceanothyn left in some doubt whether it acted directly on blood, or was made effective by gastrointestinal or liver activity. The assays described here do not exclude actions at these sites, but do show that such action is not essential for a coagulant effect. The demonstration of a diphasic effectiveness with concentration in blood is believed to explain at least one reason for previous variant reports regarding its quantitative effectiveness. The fact that Ceanothyn closely follows the action of EDTA with progressive concentration in blood strongly suggests, but does not conclusively show, that Ceanothyn acts on the calcium in blood. Direct calcium antagonism should be demonstrated by experimentation, and such experiments are in progress. Ceanothyn is known to contain dicarboxylic acids, and include oxalate.

Since EDTA is an established chelator of calcium it was considered of interest that when small amounts of calcium were chelated in blood there was a distinct coagulant action, and that this action was demonstrated *in vitro*. Within the narrow limits of the optimal concentration of EDTA this action was not negligible, but evidenced a −38% decline in the clotting time of blood. This would indicate that calcium inhibitors used to prevent blood clotting should not be used in insufficient amounts, or that none should be used that might lose its activity with time with blood or blood component storage.

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Hemopoietic Effects of Ferriferrocobaltochloroaminoacetate (L. B. 101) a Cobalt-Iron Complex*

By H. R. MURDOCK, Jr., and J. W. FISHER†

A cobalt, iron, and amino acetic acid complex was prepared and shown to be a true complex rather than a mixture of metallic glycinates. Biological utilization and easy availability of the cobalt and iron components was proved by administering the complex to rats under various experimental conditions. The hematopoietic response was compared to that of equivalent doses of cobalt chloride and ferrous sulfate and was found to be of the same order of magnitude. The toxicity of the complex is no greater than a mixture of the simple salts.

INORGANIC divalent cobalt compounds are recognized as stimulants to erythropoiesis and hemoglobinogenesis. Following the work in this country by Kato (1) many reports concerning therapeutic use of cobalt in anemia have appeared in the literature. Although cobalt forms numerous "complexes," in which the cobalt is usually in the trivalent state, reports concerning pharmacologic action of such compounds have been infrequent. This paper presents data concerning certain pharmacologic properties of ferriferrocobaltochloroaminoacetate (L. B. 101). This "complex" represents a molecular combination containing cobalt, iron and amino acetic acid and has not previously been studied. Practical utility of this complex compound would depend upon: (*a*) ability of the cobalt component to produce erythropoietic stimulation, (*b*) availability of the iron, and, (*c*) a low level of toxicity.

EXPERIMENTAL METHODS

Adult male rats of the Wistar strain maintained on a standard laboratory diet were used throughout this investigation. Control and experimental animals for each part of the study were maintained under identical conditions. Blood for the hematologic studies was obtained from the tail under partial ether anesthesia. Erythrocyte counts were done using standard pipets and a "bright-line" counting chamber. Hemoglobin determinations were done by the Sahli method.

For oral administration, except as otherwise indicated, cobalt compounds were given to rats through metal stomach tubes in an aqueous solution containing 2 mg. elemental cobalt per ml. and in instances where iron was given the concentration was 8 mg. elemental iron per ml. The concentration of cobalt solutions used for intraperitoneal administration provided 1 mg. elemental cobalt per ml. The daily oral dose of cobalt given was usually 10 mg. of the elemental cobalt per Kg. body weight, and where iron was given the dose was 40 mg. elemental iron per Kg. The intraperitoneal dose was 2.5 mg. cobalt per Kg. Exceptions to these dosages are noted in the text. All injections were made on the first five consecutive days of each week and references to daily injection refer to this schedule.

Urinary cobalt was determined by a modification of a method described by Weissbecker (2). This latter determination, as modified by us, is based upon the spectrophotometric estimation of the color appearing in cyclohexanone extracts of samples treated with potassium thiocyanate.

Chemically, ferriferrocobaltochloroaminoacetate (L. B. 101) is described as a cobalt "complex." The compound was first prepared in our laboratory by the chemical interaction of cobaltous chloride hexahydrate, ferric chloride hexahydrate, and amino acetic acid. In the complex, iron is present partially in the ferrous form. Chemical and physical investigation indicates that the molecular structure is a true complex rather than a mixture of metallic glycinates. Because the complex appears to be a loose molecular combination similar perhaps to the iron "scale salts," the molecule can be prepared to contain from 4 to 8 times as much elemental iron as cobalt. Except where otherwise indicated the preparation containing 4 times as much iron as cobalt was used. L. B. 101 is a grayish-green hygroscopic solid which tends to darken on exposure to air. It is very soluble in water but insoluble in most organic solvents. It does not precipitate in artificial gastric juice.

RESULTS AND DISCUSSION

Toxicity.—Both oral and intraperitoneal acute toxicity of L. B. 101 were determined in the adult rat. The oral LD_{50} was found at a dosage level representing 36.5 mg. per Kg. elemental cobalt and the intraperitoneal LD_{50} represented 8.4 mg. elemental cobalt per Kg. These values were of the same order of toxicity as those found with an equivalent mixture of cobalt chloride and ferrous sulfate under identical experimental conditions.

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The authors wish to express their appreciation to Joseph B. Vaughan and Dr. Harry M. Leyland for the preparation of and chemical studies on the cobalt complexes and to Dr. L. J. Klotz for assistance in preparing the manuscript.

TABLE I.—AVERAGE BLOOD VALUES AND WEIGHT CHANGES OF NORMAL ADULT MALE RATS AFTER 26 WEEKS OF A DAILY ORAL DOSE OF L. B. 101, COBALT CHLORIDE OR A SIMPLE MIXTURE OF COBALT CHLORIDE AND FERROUS SULFATE

Compound[a]	Oral Dose, mg./Kg. B. W. Co[b]	Fe[b]	Erythrocytes, millions/cu. mm. Initial	26 Wks.	Hemoglobin, Gm./100 ml. Initial	26 Wks.	Body Wt., Gm. Initial	26 Wks.
Control	0	0	9.6 ± 0.33[c]	10.3 ± 0.14[c]	17.9 ± 0.29[c]	17.3 ± 0.92[c]	271	445
L. B. 101	10	40	8.1 ± 0.46	12.6 ± 0.96	15.6 + 0.71	25.1 + 0.85	296	406
$CoCl_2$ and $FeSO_4$	10	40	8.4 ± 0.47	12.5 ± 0.81	16.6 ± 0.71	24.7 ± 0.22	268	396
$CoCl_2$	10	0	8.0 ± 0.52	12.7 ± 0.36	16.9 ± 0.44	25.1 ± 0.71	294	397
L. B. 101	5	20	7.9 ± 0.92	10.8 ± 0.68[d]	15.0 ± 0.50	21.0 ± 1.1[d]	278	426[d]
$CoCl_2$ and $FeSO_4$	5	20	7.9 ± 0.48	10.0 ± 0.16[d]	16.5 ± 0.33	19.4 ± 0.68[d]	278	383[d]

[a] Four animals were used in control group and 8 in each of the other groups.
[b] Dosage given as elemental cobalt and iron.
[c] Standard error of mean = $\sqrt{\Sigma d^2/N(N-1)}$.
[d] Followed for 17 weeks only.

TABLE II.—AVERAGE HEMOPOIETIC RESPONSE AND WEIGHT CHANGES IN ADULT MALE RATS FOLLOWING INTRAPERITONEAL INJECTIONS OF SEVERAL COBALT COMPOUNDS

Compound	No. of Rats	Dose, mg. Co/Kg.	Erythrocytes, million/cu. mm. Initial	14 Wks.	Hemoglobin, Gm./100 ml. Initial	14 Wks.	Wt., Gm. Initial	14 Wks.
Control	3[a]	0.0	8.2	9.5	17.7	17.6	206	340
$CoCl_2$	3	2.5	6.8	15.1	16.3	23.7	204	276
Co amino acetate	3	"	7.3	15.4	16.3	25.8	206	227
Co milk peptonate	3	"	7.8	15.2	16.3	24.1	211	225
Co EDTA[b]	3	"	7.5	12.4[c]	16.0	19.7[c]	211	321

[a] One rat died at end of 22 days as a result of a respiratory infection.
[b] EDTA—Ethylenediamine tetra-acetic acid.
[c] Value at the end of 9 weeks.

Hemopoietic Effect.—To determine the hemopoietic effectiveness of the cobalt component of L. B. 101, hematologic response in normal rats was followed and compared with that produced by cobalt chloride or by a mixture of cobalt chloride and ferrous sulfate in equivalent dosages. This was a satisfactory method of evaluation because cobalt possesses the unique property of producing a true polycythemia in the rat (3, 4).

The results are given in Table I. At the end of 26 weeks treatment [approximately 17% of the life span of the rat (5)] with L. B. 101 at the higher dosage level, the mean hemoglobin increase was 60.9%. With an equivalent mixture of cobalt chloride and ferrous sulfate, it was found to be 48%, and with cobalt chloride, 48.5%. Initial and final hematologic values are reported only for animals on which the study was completed. Mechanical injury due to repeated insertion of the metal stomach tube used caused the loss of some animals during the study.

Prolonged administration of relatively large doses of L. B. 101 produced the characteristic cobalt polycythemia, and was at least as effective in this way as was cobalt chloride itself or cobalt chloride given with the same amount of iron as was present in the complex (Table I). It is apparent therefore, that cobalt chemically bound in this manner is as effective a hemopoietic stimulant as are inorganic cobalt salts.

The parallel increase in hemoglobin in the group receiving cobalt chloride, as compared to that receiving ferrous sulfate in addition, suggests that the rats receiving the cobalt chloride alone were able to obtain the necessary iron either from the diet or from body stores. Dorrance, *et al.* (6), have shown increased iron absorption in rats receiving cobalt chloride.

The animals receiving the lower dosage levels (5 mg. cobalt and 20 mg. iron) (Table I) were followed for 17 weeks. It is interesting to note that in this case the hemopoietic response to the complex was as high as that obtained with an equivalent mixture of cobalt chloride and ferrous sulfate. These lower doses are still considerably higher, on a weight basis, than those used in human therapy since they represent approximately 20 to 25 times the latter dosage levels. On each successive blood sampling there was a steady rise in hemoglobin and erythrocyte values for the rats on both the higher and lower dosages. Histologic examination of the bone marrow of these rats showed that active erythropoiesis was present.

It is not surprising that the cobalt component of this molecule is effective as a hemopoietic stimulant since preliminary studies in our laboratory indicate that cobalt chelated with amino acetic acid or complexed with protein hydrolyzates is also effective (Table II). In addition, Meissner (7) reported effective hemopoiesis with "cobalt amino acids," and Dittrich (8) reported an improvement in the blood picture with a cobalt chlorophyllin compound,

TABLE III.—HEMATOLOGIC VALUES AND WEIGHT CHANGES INDUCED BY ORAL ADMINISTRATION OF L. B. 101 IN ADULT MALE RATS MADE ANEMIC BY TURPENTINE INJECTIONS

	No. Rats	Dosage, mg. Co/Kg.	Erythrocytes, million/cu. mm. Initial	51 Days	Hemoglobin, Gm./100 ml. Initial	51 Days	Body Wt., Gm. Initial	63 Days
Control	4	0	8.1 ± 0.70	9.2 ± 0.46	17.1 ± 0.88	17.7 ± 0.86	271	341
L. B. 101	6	10	8.0 ± 0.38	10.6 ± 0.33	15.8 ± 0.45	21.1 ± 0.68	209	306
L. B. 101[a] turpentine injections	6	10	7.4 ± 0.48	10.4 ± 0.72	15.1 ± 0.40	19.1 ± 0.56	209	257
Turpentine injections alone	6	0	7.7 ± 0.20	8.8 ± 0.19	15.4 ± 0.20	14.8 ± 0.46	214	271

[a] One animal died the first week under ether anesthesia.

Although the hemopoietic response described above would seem to be quite conclusive evidence that cobalt in L. B. 101 has been absorbed and had exerted a hemopoietic effect, it was felt desirable to demonstrate that absorption had occurred. This was done by demonstrating the appearance of cobalt in the urine in the following manner. One group of three rats was given orally a total of 12.4 mg. of elemental cobalt in the form of L. B. 101 and another group of three rats received cobalt chloride at the same cobalt dosage level. The urine from each group was collected over a 22 hour period, pooled, and total cobalt determined as described earlier. The rats receiving the L. B. 101 excreted 0.87 mg. cobalt, and the rats receiving cobalt chloride excreted 0.64 mg. This is 5–7% urinary excretion, which is similar to that reported by Comar, *et al.* (9), and is further proof of the gastrointestinal absorption of the cobalt in L. B. 101.

Systemic absorption after intraperitoneal administration was also demonstrated in the same manner. A collective five-hour urine sample from three rats receiving a total intraperitoneal dosage of 1.5 mg. cobalt as L. B. 101 showed a total excretion of 0.45 mg. elemental cobalt. Three animals receiving cobalt chloride in the same dosage also showed a corresponding elimination of 0.45 mg. These excretion values represented approximately 33% of the injected dose.

Effect on Depressed Hemopoiesis.—As further evidence of the effectiveness of the cobalt component of the L. B. 101 complex, the effect in overcoming the inhibition of the hemopoietic system produced by turpentine injection was demonstrated.

It was felt that this was a desirable method of investigation because sterile turpentine abscesses in animals produce a hemopoietic result similar to that seen in chronic infection in humans. Cobalt has been widely used therapeutically to combat anemia due to infection of chronic suppurative diseases (10). The technique was that used by Wintrobe, *et al.* (11), who showed that administration of cobalt chloride could prevent the anemia in the rat caused by turpentine induced abscesses. The rats received 0.25 ml. U. S. P. turpentine in the posterior thigh muscle once or twice a week as required to maintain the abscess. The results (Table III) are similar to those reported by Wintrobe, *et al.* (11). The turpentine injections prevented the normal expected rise in hemoglobin and erythrocytes seen in the control group of rats and also obtained in other studies in our laboratory. When L. B. 101 was given along with the turpentine, the blood values increased to a level similar to those obtained from L. B. 101 injections alone; thus, the cobalt in L. B. 101 must have been available to exert its hemopoietic action.

Availability of Iron Component in Ferriferrocobaltochloroaminoacetate.—Previous reports have shown that pure iron deficiency can be produced in rats maintained on a milk diet. The fact that hemopoietic depression is not a factor is indicated by the prompt response to iron administration (12). Cobalt alone, therefore, could have no appreciable influence on such an anemia.

This type of anemia was produced in adult male rats by placing them on a strict milk diet and withdrawing blood from the heart once or twice a week until hemoglobin values failed to rise between bleedings. The iron stores were assumed to be depleted when hemoglobin did not increase during the three weeks following the last heart puncture. Erythrocyte values in these animals were not greatly lowered and could not be further depressed because of the condition of the animals. The rats, averaging 325 Gm. body weight, were divided into three groups at random. Group I (5 rats) was given L. B. 101 prepared to contain approximately six times as much iron as cobalt. The daily oral dose provided 0.2 mg. elemental cobalt per Kg. and 1.3 mg. elemental iron per Kg. Group II (5 rats) was given only ferrous sulfate to provide the same oral dosage of elemental iron mentioned above. Group III (2 rats) was used as the control. All animals received 0.2 mg. per Kg. of elemental copper orally to meet the dietary requirement of copper, and the milk diet was continued throughout the experiment. The dosage of cobalt used in this experiment corresponds approximately, on a weight basis, to that commonly used in therapeutics.

Results are shown in Fig. 1. Hemoglobin values for both medicated groups increased in a parallel manner throughout the period of treatment. Subsequently, however, the cobalt-iron treated animals achieved somewhat higher values than did those receiving iron alone.

It is of interest to note that this chart is strikingly similar to that of Rohn, Bond, and Klotz (13) repre-

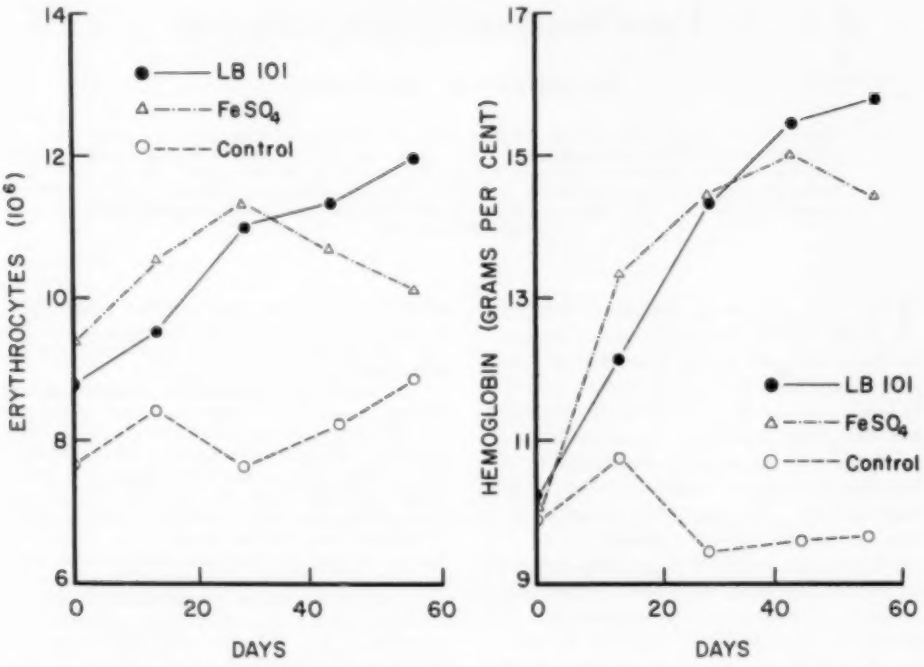

Fig. 1.—Average rise in erythrocytes and hemoglobin values in iron-deficient (milk-anemic) rats given L. B. 101 as compared with ferrous sulfate.

senting the response to cobalt in human iron-deficiency anemia and may be a further indication of the ability of cobalt to maintain hematopoiesis at the maximum rate irrespective of decreasing demand as normal levels are approached. The slope of the erythrocyte curves in this experiment is similar to those obtained in normal rats fed 10 mg. of elemental cobalt per Kg.

SUMMARY

1. The cobalt component of the complex compound, ferriferrocobaltochloroaminoacetate, is as effective a hemopoietic agent as are inorganic cobalt and iron in stimulating hemopoiesis or in overcoming hemopoietic inhibition in the rat. Effects are comparable either on oral or intraperitoneal administration and excretion data indicate comparable absorption rates.

2. The iron component of the complex compound, ferriferrocobaltochloroaminoacetate, is absorbed after oral administration and is utilized to approximately the same degree as is iron given as ferrous sulfate in the iron deficient rat.

3. The toxicity of ferriferrocobaltochloroaminoacetate prepared to contain four times as much elemental iron as cobalt is approximately the same as that of an equivalent dosage of a mixture of ferrous sulfate and cobalt chloride.

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In Vitro Study of Gastric Antacids*

By GEORGE M. NAIMARK

Ten antacid preparations were evaluated, using the Holbert modification (4) of the Johnson and Duncan procedure (3) and simulating typical *in vivo* dosages and physical forms. Several of the antacids rapidly raised the pH of gastric juice to a point above that usually considered desirable while one preparation raised the pH to a maximum of 3.7. The importance of the antacid dosage form in releasing the active ingredients is stressed.

THE NEED FOR gastric antacids to lower the acidity of the stomach contents continues unabated since Paracelsus prescribed powdered pearls and Pliny commended crushed coral (1). Although the use of such agents by physicians and the laity continues to increase, much work has yet to be done in evaluating the characteristics of the many available antacid materials (2). The successful evolution of *in vitro* procedures, closely simulating *in vivo* conditions, has provided a practical technique for comparative study of antacids under standardized and reproducible circumstances. This refinement in methodology suggested the following procedure as one that closely parallels intragastric conditions: the antacid is added in usual dosage and in appropriate physical form to synthetic gastric juice, maintained at body temperature and stirred continuously at a constant rate; portions of the reaction mixture are removed at definite intervals (to simulate periodic emptying of the stomach) and replaced with equivalent volumes of fresh gastric juice also maintained at body temperature (to simulate continual secretion of gastric juice); pH changes are checked throughout the test. This is essentially the method of Johnson and Duncan (3) which has been used with modification by many workers (4–8). A useful modification of the method (4) was selected by us for comparative evaluation of miscellaneous antacid preparations, including several that had not previously been studied by this procedure.

EXPERIMENTAL

Test Method.—The Holbert modification (4) of the Johnson and Duncan procedure (3) was used.

Artificial Gastric Juice.—The U. S. P. XV artificial gastric juice was used with pH adjusted to 1.6 to approximate closely the initial pH utilized by Holbert and co-workers (4, 5, 8).

Antacid Addition.—It was considered practical to adhere to typical dosages and to introduce the antacid materials into the artificial gastric juice in physical forms that would correspond to *in vivo* use. The dosage and physical state of each antacid product are summarized in Table I.

Criteria of Effectiveness.—In clinical use, an antacid preparation should have the ability to raise and maintain the pH of the gastric contents within a range sufficiently high to control the symptoms of hyperacidity, but not so high as to result in the possibility of acid rebound. Dale and Booth (9) have recently reviewed the lack of agreement concerning this desired pH range. Nonetheless, in our work we have been guided by the opinion expressed in New and Nonofficial Remedies (10) to the effect that effective neutralization involves raising the pH to 4.0 or 5.0, and that of Kirsner and co-workers (11): "Effective neutralization may be defined as maintenance of the pH between 4.0 and 5.5 or higher." In the evaluation of antacids in our laboratory, a pH range of 4.0 to 6.0 has been selected as the desired range.

RESULTS

The results are graphically shown in Figs. 1 and 2. Each curve represents an average of duplicate determinations for each antacid studied.

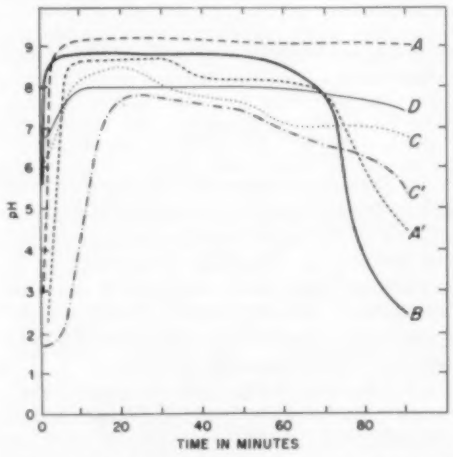

Fig. 1.—Neutralization characteristics of samples A through D.

* Received May 25, 1956 from Medical Research Department, White Laboratories, Inc., Kenilworth, N. J.

TABLE I

Antacid Product	Dosage Used in Evaluation	Physica State of Antacid as Used in Test	Sample Designation
Magnesium hydroxide tablets	2 tabs. (1.2 Gm.)	(1) Ground tabs. (2) Intact tabs.	(1) A (2) A′
Magnesium hydroxide suspension Magnesium trisilicate Calcium carbonate	1 tsp.	As is	B
Magnesium hydroxide tablets Sodium bicarbonate Magnesium carbonate Bismuth subnitrate	2 tabs. (1.2 Gm.)	(1) Ground tabs. (2) Intact tabs.	(1) C (2) C′
powder	1 tsp.	As is	D
Magnesium glycinate powder	900 mg. (450 mg. initially, 450 mg. after 2 min.)	As is	E
Sodium bicarbonate powder Calcium carbonate Magnesium carbonate	½ tsp.	As is	F
Magnesium trisilicate tablets Calcium carbonate Magnesium trisilicate	2 tabs. (2.7 Gm.) 305 mg. calcium carbonate and 145 mg. magnesium trisilicate	Ground tabs.	G
Chewing gum tablet	initially, repeated after 2 minutes[a]	As is	H
Calcium carbonate Magnesium carbonate Magnesium trisilicate tablets	2 tabs. (2.4 Gm.)	Intact tabs.	I
Dihydroxy aluminum sodium carbonate tablets	2 tabs. (3.0 Gm.)	Ground tabs.	J

[a] Simulating actual release of ingredients from two tablets.

Under the conditions of this study, the antacid preparations depicted in Fig. 1 acted rapidly but tended to raise and maintain the pH within an alkaline range and thus, in clinical use, may possibly induce acid rebound. Also noteworthy are the differences in rapidity of action, maximum pH achieved, etc. when the physical state of a given antacid was altered. For example, with intact tablets (samples A′ and C′), the maximum pH was less and the time required to attain this pH greater than with tablets that had been comminuted (samples A and C) before addition to the gastric juice.

With the exception of samples H and J most of the antacid preparations for which results are shown in Fig. 2 raised the pH of the artificial gastric juice to a level which might result in acid rebound. Sample J, which had been added to the reaction mixture in the form of ground tablets, maintained the pH above 3.5 for 27 minutes while achieving a maximum pH of 3.7.

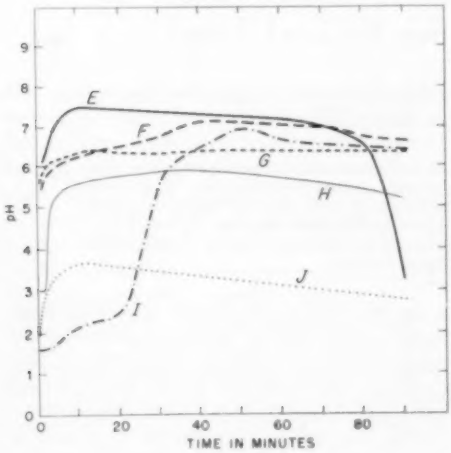

Fig. 2.—Neutralization characteristics of samples E through I.

DISCUSSION

Generally speaking, most of the antacids tested rapidly brought the pH to a point above that usually considered desirable. Sample J maintained the pH above 3.5 for 27 minutes and never raised the pH to an objectionably high level. Sample H, under the conditions of this study, kept the pH between 5.0 and 6.0 for most of the test period.

The results depicted in Fig. 1 on the antacid activity of different physical forms of the same formulation serve to emphasize the importance of the mode of administration of the antacid materials to the stomach. Kirsner and Palmer (12) emphasized that magnesium trisilicate in powder form was more effective than as tablets because of better interaction between the antacid and the free hydrochloric acid. Goodman and Gilman (13) also stress that a large surface area is necessary for effective action of magnesium trisilicate. Similarly, Rossett and Rice

(14), have shown that the liquid preparations of aluminum hydroxide are much more reactive than the tablet form, also presumably because of the increased surface area available when the antacid is suspended in a liquid medium. Obviously, then, it is an oversimplification to evaluate an antacid formulation solely through consideration of its active constituents. The success of an antacid product is equally dependent upon the ability of the dosage form to yield the active ingredients at the proper rate and with the maximum possible surface area. If a tablet, or pieces of a tablet, disintegrate slowly in the stomach, release of the antacid ingredients may be too slow for effective neutralization or may even pass into the intestine before completely reacting with the gastric acid. In this connection, Kirsner and Palmer (15), state that, in their experience, the neutralizing value of any antacid in tablet form is distinctly inferior to that of powder or liquid preparations, because of passage of the former into the intestinal tract before reaction is complete. It appears to be essential, therefore, in antacid formulation or evaluation, to give consideration to the dosage form of the material and to its ability to release its active constituents at the desired rate and in the most active therapeutic physical form.

SUMMARY

1. Ten antacid products were tested for antacid effectiveness using the Holbert (4) modification of the Johnson and Duncan procedure (3) and simulating typical dosages and usual *in vivo* modes of administration.

2. The majority of the antacid materials rapidly raised the pH of gastric juice to a point above that usually considered desirable. One sample maintained the pH above 3.5 for 27 minutes while reaching a maximum pH of 3.7. Another product kept the pH between 5.0 and 6.0 for most of the test period.

3. The importance of the antacid vehicle was discussed from the standpoint of rate of release and physical form of the active ingredients.

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Hypotensive Activity of a New Ganglionic Blocking Agent and Its Comparative Effectiveness on Normotensive Unanesthetized Dogs*

By W. EDWARD O'MALLEY, GISELA WINKLER, LEONARD M. RICE, and CHARLES F. GESCHICKTER

Methodology was established for comparison of hexamethonium, pentolinium, and H-2 for their vasodepressive responses on intact, unanesthetized dogs. Comparisons were made at various dosage levels. Intravenous toxicities on dogs and intraperitoneal toxicities on rats were also performed for the three agents. It was found that H-2 was moderately more potent and markedly less toxic than either hexamethonium or pentolinium.

WITH THE INTRODUCTION of hexamethonium in 1948 by Paton and Zaimis (1) it became possible to significantly deter the progress of hypertensive vascular disease, especially the malignant variety, by the use of pharmacologic agents. The value of hexamethonium was confirmed by Restall and Smirk (2) in New Zealand, Campbell and Robertson (3) in Great Britain, and Finnerty and Freis (4) in the United States.

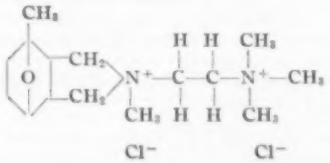
Wien and Mason (5) and Smirk (6) in 1953 introduced pentolinium, a ganglionic blocking agent chemically and pharmacologically similar to

* Received August 18, 1956 from the Georgetown University Medical Center, Department of Pathology, Washington 7, D. C.
Supported by a research grant for the Geschickter Fund for Medical Research, Inc.

hexamethonium, but possessing properties of greater potency, more prolonged activity, and more dependable absorption. The work of Freis and co-workers (7,8) and Gifford and co-workers (9) have further established the value of this agent in hypertension.

A new unsymmetrical bis-methonium compound was submitted to us for evaluation,[1] since preliminary screening indicated ganglionic blocking activity, combined with a low degree of toxicity. This claim was attractive, for none of the currently available ganglionic blocking agents possesses low toxicity together with hypotensive properties.

The structure of the foregoing compound is shown below. It was synthesized and previously reported by Rice (10). It shall hereafter be referred to as H-2.



It is the purpose of this communication to present a laboratory comparison of the agent H-2 with hexamethonium and pentolinium in respect to its hypotensive activity and its acute toxicity. Since the validity of comparisons is dependent upon the reliability of the procedure used, several attempts were made to establish a reliable method.

Intact Rats.—Pressure measurements on the intact rat by the method of Kersten, *et al.* (11), were unreliable in our hands. Moreover, it is our belief that the dog is a better test object, for by virtue of its size, symptomatology can be observed better than on the rat.

Normotensive, Anesthetized Dogs.—The vast majority of laboratory experiments have been performed, using anesthetized dogs as test objects. Two facts made us abandon this method: First, the duration of the experiments is limited, for the anesthetized animals sooner or later show varying degrees of shock. Second, depression of the reticular formation in the medulla by anesthesia potentiates the vasodepression of hypotensive agents.

Normotensive, Pre-anesthetized Dogs.—Trapold, *et al.* (12), studying Serpasil,® attempted to avoid the anesthetized preparation by inserting a catheter into the brachial artery under Pentothal.® The dog was permitted to recover from the anesthesia before proceeding with the hypotensive studies. In our experience, postoperative pain and trauma markedly alter normal blood pressure patterns.

To obviate the foregoing difficulties, the authors have used a modification of the procedure of Dameshek and Loman (13) for measuring the blood pressure. Employing the methodology as presented below, pentolinium bitartrate, hexamethonium chloride, and H-2 chloride were compared for their hypotensive activities and acute toxicity.

METHODS

Blood Pressure Measurement—The blood pressures of unanesthetized dogs were recorded by puncturing the femoral artery with a 20-gauge needle just distal to the origin at the inguinal ligament. The two side arms of an interposed three-way stopcock served to connect an anticoagulant-filled rubber tube to a mercury manometer which recorded the mean systolic blood pressure. Dogs were placed on their backs upon an operating table for puncture. This must be done with the utmost gentleness, otherwise excitement and fear will make the measurements unreliable. With patience and practice in this matter one is able to take consecutive readings which vary only within a 5-mm. range. In order to allow the animals to become accustomed to this procedure and to establish a base-line for each dog, blood pressure measurements were taken preceding the test period several times a week for four weeks on nine male dogs weighing 12.0 to 14.0 Kg. These dogs had been previously selected from a larger group because of gentleness and tractability. The record kept on each dog showed that during this educational period the initial blood pressure readings were higher than normal and finally declined to a steady lower level prior to the end of this four-week period.

The trained dogs were divided into three groups of three dogs each. Group I represented the highest dosage level of 50 mg./Kg.; Group II represented an intermediate dosage level of 20 mg./Kg.; and Group III represented the lowest dosage level of 10 mg./Kg. In calculating the dosage of the drug milligrams of active cation were employed. Each of the three dogs in Group I received 50 mg./Kg. of hexamethonium. After two weeks the same three dogs then received 50 mg./Kg. of pentolinium, and, after another 2-week interval, 50 mg./Kg. of H-2. This 2-week interval was permitted to pass between the administration of the different compounds in order to get adequate recovery from the drug effects. This method permitted a comparison of the three drugs on the same dog. The dogs in Group II and Group III were treated similarly, except that they received 20 mg./Kg. and 10 mg./Kg of the three compounds, respectively. All of the drugs were administered orally in aqueous solution by stomach tube. No two dogs received the same order of administration of the three separate hypotensive agents.

An initial blood pressure reading was taken on each dog just before the administration of the drug. Then the effect of the drug on the blood pressure was

[1] This compound is presently available from Wyeth Laboratories, Inc., for experimental and clinical investigation as Wy-1263.

TABLE I.—MEANS AND STANDARD ERRORS OF BLOOD PRESSURE MEASUREMENTS

Time After Drug Administration	Group I (50 mg./Kg.) Hexamethonium	Pentolinium	H-2	Group II (20 mg./Kg.) Hexamethonium	Pentolinium	H-2	Group III (10 mg./Kg.) Hexamethonium	Pentolinium	H-2
0 Minutes	132 ± 3	132 ± 4	134 ± 4	129 ± 6	130 ± 6	129 ± 5	125 ± 5	129 ± 5	132 ± 7
30 Minutes	108 ± 4	109 ± 5	119 ± 2	108 ± 5	113 ± 6	108 ± 7	98 ± 3	121 ± 8	114 ± 9
1 Hour	106 ± 4	101 ± 5	101 ± 5	112 ± 5	107 ± 8	86 ± 5	97 ± 6	114 ± 9	105 ± 6
1 Hr. 30 min.	107 ± 4	95 ± 5	98 ± 6	112 ± 4	90 ± 4	86 ± 4	100 ± 6	108 ± 10	99 ± 8
2 Hours	110 ± 4	97 ± 3	99 ± 7	120 ± 5	95 ± 5	89 ± 5	101 ± 6	107 ± 10	98 ± 7
4 Hours	115 ± 5	108 ± 4	96 ± 5	130 ± 6	105 ± 4	94 ± 5	106 ± 6	105 ± 9	102 ± 9
6 Hours	117 ± 8	110 ± 5	92 ± 5	130 ± 6	108 ± 7	98 ± 5	116 ± 5	110 ± 9	109 ± 8
9 Hours	117 ± 7	102 ± 4	95 ± 3	132 ± 6	102 ± 9	98 ± 5	119 ± 4	117 ± 8	118 ± 9
12 Hours	124 ± 9	99 ± 4	100 ± 5	129 ± 6	116 ± 10	108 ± 4	120 ± 4	123 ± 8	122 ± 9
15 Hours	128 ± 5	102 ± 6	101 ± 6	129 ± 6	123 ± 8	108 ± 6	122 ± 4	122 ± 9	127 ± 6
18 Hours	130 ± 5	107 ± 7	104 ± 6	129 ± 6	129 ± 6	114 ± 4	123 ± 6	125 ± 7	126 ± 7

determined by readings taken at 30-minute intervals for the first two hours, and then at 4, 6, 9, 12, 15, and 18 hours after the time of the initial administration.

Acute Toxicity.—Acute toxicities were performed at various dosage levels by injecting intravenously each agent at a uniform rate over a 6-minute period. Pentolinium bitartrate and hexamethonium chloride were administered in the form of a 4.0% solution on the basis of cation. Owing to the low toxicity of H-2 chloride, it was found necessary to utilize a 10.0% solution of H-2 cation. During the intravenous titration the dogs were not anesthetized. To avert the possibility of withdrawal of the needle during the intravenous injection period, a polyethylene catheter was inserted deeply through a 15-gauge needle previously placed into the great saphenous vein.

Twenty-four hour LD_{50}'s were determined on white male rats of the Wistar strain, 180–220 Gm., by the method of Litchfield and Wilcoxon (14).

RESULTS

Blood Pressure Measurements.—In order to avoid unnecessary description the data are presented in graphic form. A curve was plotted for each dosage group and the initial pressures of the three dogs belonging to the same group were averaged. Also averaged were the results taken at the various intervals after drug administration. Each curve which presents readings over 18 hours is therefore an average of one group's blood pressure responses to a particular agent at a particular dosage level. This was done to reduce the error of biologic variability. Standard error and standard deviation from the mean arterial pressure were calculated, using the original measurements and applying the following formulas:

$$\text{S. D.} = \pm\sqrt{\text{Ed}^2/(n-1)} \qquad \text{S. E.} = \pm\,\text{S. D.}/\sqrt{n}$$

Figure 1 illustrates the effect of 50 mg./Kg. of hexamethonium, pentolinium, and H-2 on the dogs in Group I. This represents the highest dosage level. Figure 2 illustrates the effect of 20 mg./Kg. of hexamethonium, pentolinium and H-2, respectively, on the Group II dogs which represent the middle dosage level. Figure 3 illustrates the effect of 10 mg./Kg. of hexamethonium, pentolinium, and H-2 on Group

TABLE II.—SIGNIFICANT VASODEPRESSIVE RESPONSE (DEPRESSION OF 25 MM. HG OR MORE) TO HEXAMETHONIUM, PENTOLINIUM, AND H-2

Compound	Dosage Level (*per os*), mg./Kg.	Duration of Response
Hexamethonium	10	90 Min.
	20	[a]
	50	75 Min.
Pentolinium	10	[a]
	20	9 Hr.
	50	18 Hr.
H-2	10	5¼ Hr.
	20	10½ Hr.
	50	18 Hr.

[a] Blood pressure did not fall as much as 25 mm. Hg.

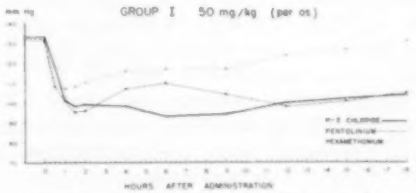


Figure 1. Hours after Administration

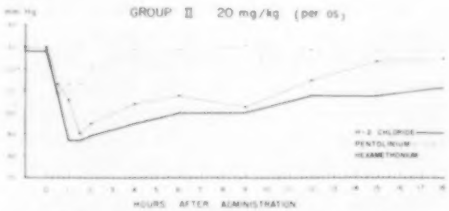


Figure 2. Hours after Administration

III dogs in the lowest dosage level. Table I summarizes the means and standard errors of the means for all three groups. Table II illustrates the effect of the three ganglionic blocking agents studied in terms of significant blood pressure response, which was considered to be a depression of 25 mm. of Hg or more. The short duration of response to hexamethonium and the prolonged response to pentolinium and to H-2 should be noted.

Acute Toxicity.—The results of the acute toxicities are shown in Table III. In our experiments with unanesthetized dogs, pentolinium elicited death at the low dosage level of 10.0 mg./Kg. of cation. Hexamethonium was moderately less toxic: 35.0 mg./Kg. of cation was the approximate median lethal dose. In contrast to the above two drugs, H-2 was relatively innocuous; the approximate median lethal dose was 200 mg./Kg. of active cation. With all three agents the mechanism of death was respiratory depression.

The relative degree of toxicity found in dogs was confirmed by the 24-hour intraperitoneal LD_{50} on rats, as presented in Table IV.

DISCUSSION

Blood Pressure.—As indicated in Table II, a significant depressive response (25 mm. Hg or more), which did not appear to depend upon the dosage level, was achieved with hexamethonium for only brief intervals. Pentolinium elicited significant hypotensive reactions for prolonged periods at dosage levels of 20 mg./Kg. and 50 mg./Kg., H-2 caused significant response for prolonged intervals at dosage levels of 10, 20, and 50 mg./Kg.

Side-effects resulting from blockade of the autonomic ganglion, which regulates the eyes, were seen with all agents. Other side-effects were not observed.

Acute Toxicity.—The acute toxicity titrations indicate that intravenous administration of 10 mg./Kg. of pentolinium will produce death in normal dogs. Hexamethonium will produce death at intravenous dosage levels of 35 mg./Kg. This drug is therefore moderately less toxic than pentolinium, but still dangerous on intravenous administration. H-2, with a correspondingly lethal intravenous dosage level of approximately 200 mg./Kg., has therefore a much greater range of safety when administered by this route. It is 20 times as safe as pentolinium and 5 to 6 times as safe as hexamethonium.

The relative lack of toxicity and the potency of H-2, when taken together, appear to obviate the fundamental danger inherent in the oral use of hexamethonium and pentolinium, that of sudden intestinal absorption that may mimic intravenous use. Such capricious and rapid absorption may result in toxic effects and sometimes in death.

CONCLUSIONS

A new hypotensive therapeutic compound, an isoindole-derivative, referred to as H-2, has been evaluated against pentolinium and hexamethonium in normotensive, unanesthetized dogs. It is effective orally at the low dosage level of 10.0 mg./Kg. Its activity lasts, after the administration of 20 mg./Kg., longer than 10.5 hours. At a dosage level of 50 mg./Kg. the duration of the depressive action exceeds 24 hours. The margin of safety appears much greater than that of the other hypotensive agents with which it has been compared. The intravenous LD_{50} is approximately 200 mg./Kg.

Therefore, the following statements can be made: (*a*) H-2 is moderately more potent than hexamethonium and pentolinium, (*b*) H-2 has a duration of activity equal to or greater than pentolinium; while hexamethonium is so transient that it is not in the same category as the other two, (*c*) H-2 in comparison to pentolinium and hexamethonium, has a low toxicity and is relatively innocuous.

TABLE III.—ACUTE TOXICITY OF HEXAMETHONIUM, PENTOLINIUM, AND H-2 BY INTRAVENOUS TITRATION

Compound	Number of Dogs	Dosage, Levels[a] of 6 Minutes i. v. Injection, mg./Kg.	Number of Deaths	Number of survivors	Estimated Medium Lethal Dosage, mg./Kg.
Hexamethonium 4% solution	2	30	0	2	
	2	40	2	0	35
	2	70	2	0	
Pentolinium 4% solution	2	8	0	2	
	4	12	3	1	
	1	14	1	0	10
	2	16	2	0	
	1	19	1	0	
H-2 10% solution	2	170	0	2	
	4	200	2	2	200
	2	250	2	0	

[a] Dosage levels were calculated on the basis of cation.

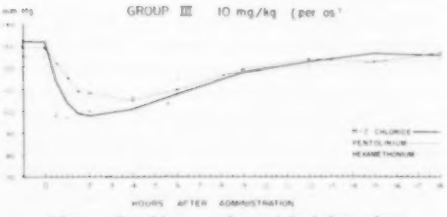


Figure 3. Hours after Administration

TABLE IV.—TWENTY-FOUR-HOUR I. P. LD_{50} RATS

Compounds	24-Hr. I. P. LD_{50} Rats, mg./Kg.	Confidence Limits (95%), mg./Kg.
Hexamethonium	100	79.4–120.6
Pentolinium	28.9	24.7–33.1
H-2	>1,000	

SUMMARY

A method of quantitatively comparing hypotensive agents has been presented. Hexamethonium, pentolinium, and a new isoindole derivative, H-2, have been compared. H-2 has been observed to possess excellent hypotensive propties, exceeding those of hexamethonium and pentolinium. It was decidedly less toxic than the latter agents.

ADDENDUM

Preliminary clinical studies by Dr. Edward D. Freis, Department of Medicine, Georgetown Medical Center, have demonstrated ganglionic blocking activity and hypotensive potency in man both by parenteral and oral routes of administration.

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Sorption of Water by Rubber Closures for Injections II.*

Effects of Vapor Pressure, Bisulfite, and Washing Treatments

By GEORGE MILOSOVICH† and ALBERT M. MATTOCKS

The effect of vapor pressure lowering, bisulfite, and washing procedures on the sorption of water by rubber closures has been studied. A logarithmic relationship between sorption of water by rubber closures and depression of vapor pressure due to inorganic salts was derived. An apparatus suitable for vapor pressure measurements of solutions is described. Sulfur dioxide was shown to be the cause of the increased sorption exhibited from bisulfite solutions. The attempt to evaluate washing procedures by means of sorption measurements was not successful.

IN A PREVIOUS PUBLICATION (1) the effect of various inorganic ions on the sorption of water by rubber closures was reported. As a result of this investigation it was concluded that vapor pressure depression caused by addition of inorganic salts was the primary factor causing a depression in the sorption of water. It was therefore thought desirable to establish the relationship between water sorption and vapor pressure. Such a relationship might serve to predict the water sorption from a solution of known vapor pressure thus enabling one to determine whether a compound has effects beyond simple vapor pressure lowering. Such effects as reaction with the rubber composition, sorption of a compound from solution by the closure or removal of material from the closure would be shown by a difference in water sorption from that predicted by vapor pressure considerations.

It was also shown that the bisulfite ion caused a marked difference in the sorptive characteristics of rubber closures. It has been reported (2–4) that rubber compositions react in some way with bisulfite causing a loss of the bisulfite from solution. Recognizing this the British Pharmacopoeia (5) requires that closures which are to be used for solutions containing bisulfite as a preservative must be soaked in bisulfite prior to use. No information as to how bisulfite reacts with rubber under these conditions could be found. However, since bisulfite does cause a marked increase in water sorption and does

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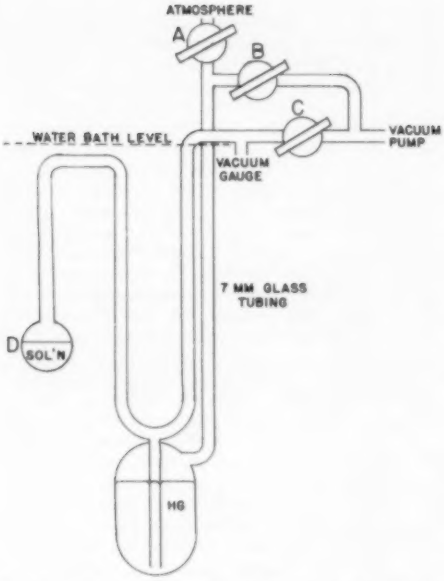

Fig. 1.—Diagram of the Vapor Pressure Apparatus.

react in some way with rubber it was felt that an investigation of this effect would give an insight into the types of reactions one might expect between components of an injection solution and the rubber closure. Such an investigation was conducted and is reported in this paper.

Numerous washing procedures or pretreatments are employed in readying the closures for use, each being claimed to aid in preventing instability. It was felt that an evaluation of these procedures may be obtained through water sorption measurements. The results of an attempt to accomplish this are also reported.

EXPERIMENTAL

The Effect of Vapor Pressure Lowering.—It has been reported (6) that no relationship between relative vapor pressure and sorption of water for rubber has been established, indicating that a simple relationship does not exist. However, it was felt that an empirical equation, though complex, could be determined which would permit calculation of the amount of water sorbed with a given vapor pressure. For the establishment of such a relationship the vapor pressure of various solutions must be known along with the sorption of closures immersed in them. Since sorption from twenty inorganic solutions was known (1), the vapor pressures of these solutions was needed. Vapor pressures of these solutions for the conditions under which the sorption experiments were conducted could not be obtained from the literature.

The Menzies isoteniscope affords a rapid means of obtaining vapor pressures but the liquid must be boiled to remove gases and such a boiling out procedure will concentrate solutions giving misleading results. To eliminate this disadvantage an apparatus was constructed in which the gases could be removed from a solution with very little loss of water vapor. This modified isoteniscope is shown in Fig. 1.

The solution to be measured was introduced into the glass bulb, D, which was then sealed to the apparatus. The solution was frozen using a mixture of dry ice in acetone, and the system was evacuated to a pressure of 0.03 mm. of mercury by closing stopcock A and opening B and C. B was then closed and A opened slowly to allow the mercury to rise in the U-tube to seal off the bulb from the vacuum pump. Then A was closed and the solution thawed, driving off gas which had been released during freezing and trapped within the ice. The solution was then refrozen, C closed and B opened to lower the mercury, opening the pathway for the freed gases. When the mercury was down in the bulb, B was closed, and as soon as high vacuum had been re-established C was opened and the freed gases were pumped off. The procedure was repeated until no gas remained in the solution, as evidenced by a constant reading after subsequent freezing and evacuation steps. To obtain a vapor pressure reading the apparatus was immersed in a transparent constant temperature water bath at 30.1° with mercury in the U-tube. After equilibrium was reached the difference in height of the mercury columns was read with a cathetometer. The temperature variation and error in reading the mercury levels were such as to give readings with a precision of one-tenth millimeter or better.

Using this apparatus the vapor pressures of the twenty inorganic solutions was determined. A plot of water sorption *versus* depression of vapor pressure is shown in Fig. 2.

The curve shown in Fig. 2 is described by the equation $10^{\frac{S}{100}} = D^{-1} + 10^{\frac{132.7}{100}}$ or in its logarithmic form, $S = -100 \log D + 132.7$, where S is the average extrapolation value among six compositions (Gm. x 10^4) and D is the depression of vapor pressure (mm. Hg).

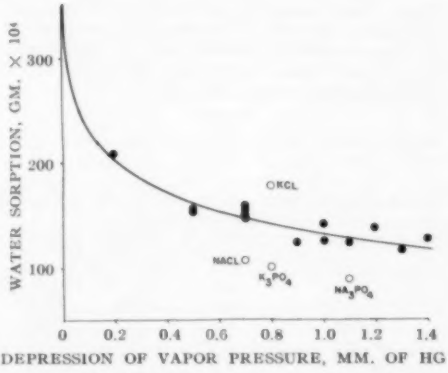

Fig. 2.—Effect of Vapor Pressure Depression on Sorption.

The constants for the equation were obtained by least squares calculation, and the standard deviation of the points on the S axis was found to be 6.3 x 10^{-4} Gm. Four values were outside the range of $\pm 3\ \sigma$ and may thus be considered to differ from the rest. The four solutions were potassium chloride (high sorption), sodium chloride (low sorption), and potassium and sodium phosphate (low sorption). It appears that these four compounds thus have an effect on sorption in addition to simple vapor pressure lowering. The low results observed with the phosphates is probably due to the basicity of the solutions since previous experiments (7) indicated that basic inorganic substances depress sorption to the greatest extent. No explanation can be advanced for the differences between sodium and potassium chloride.

Effect of Bisulfite on Sorption.—The vapor pressure of fresh 0.5 M sodium bisulfite solution was found to be 32.0 mm., while that of a three-week-old solution was 45.0 mm. This indicated that a breakdown of bisulfite was occurring with the liberation of a gas, probably sulfur dioxide. A literature search confirmed this indication, for it is apparently well known that bisulfite is unstable in solution giving rise to sulfur dioxide according to the following equation:

$$2\ NaHSO_3 \rightarrow Na_2SO_3 + SO_2 + H_2O$$

It was believed, therefore, that high sorption of closures from bisulfite solutions may be due to a sorption of sulfur dioxide as well as water. The weight of sulfur dioxide sorbed could not account for the total increase, however, and it was thought that the sulfur dioxide actually promoted water sorption. To test this hypothesis the sorption from aqueous solutions of sulfur dioxide was measured with Gum 79 and Pink 124 closures, these having been found to give the two extremes in previous sorption experiments. The sulfur dioxide solutions were prepared by dissolving the gas in water, making appropriate dilutions, and determining the concentrations by means of iodimetry.

Sorption from these solutions was found to obey the normal equation ($w = kt^n$) and the results, reported as one-year extrapolation values, are shown in Table I along with vapor pressure measurements.

The slopes of the sorption curves for each type of closure were essentially the same for all strengths of solution, the curves differing only in intercept. An exponential relationship was shown between extrapolated values and concentration by linear logarithmic plots shown in Fig. 3.

TABLE I.—RESULTS OF SORPTION FROM SULFUR DIOXIDE SOLUTIONS

SO_2 Concentration, M/L.	V. p., mm.	One-Year Extrapolation Values, Gm. × 10^6 Gum 79	Pink 124
0.190	123.5	1260	12680
0.089	65.7	1007	12000
0.066	52.8	885	12050
0.042	46.4	839	10060
0.022	37.7	697	9620
0.0065	33.1	594	8770

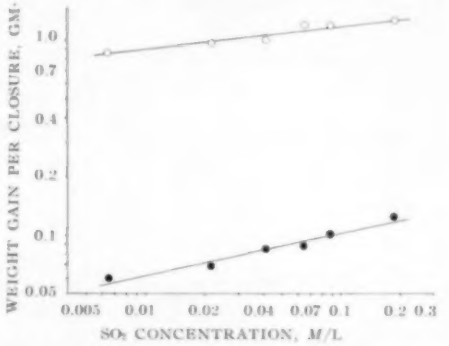

Fig. 3.—Effect of Sulfur Dioxide Concentration on Sorption. —○— Pink 124, —●— Gum 79.

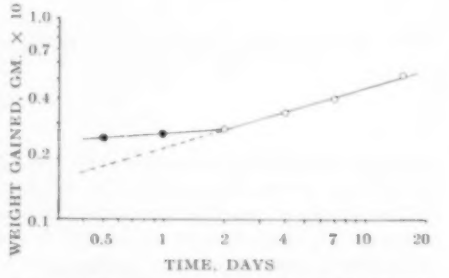

Fig. 4.—Water Sorption Following Sulfur Dioxide Treatment of Red 87 Closures. —●— Sorbed Water Plus Residual Sulfur Dioxide.

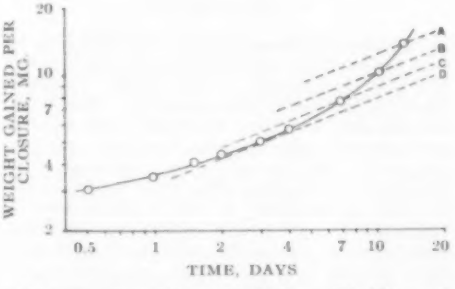

Fig. 5.—Comparison of Sodium Bisulfite and Sulfur Dioxide Curves for Gum 79 Closures. —○— 0.5 M Sodium Bisulfite, A—0.0065 M SO_2, B—0.0030 M SO_2, C—0.0016 M SO_2, D—0.0010 M SO_2.

The closures readily sorbed sulfur dioxide to the extent of 15 to 40 mg. when placed in an atmosphere of the gas. Subsequent "drying" at 60° showed that the gas was rapidly desorbed, the closures returning to initial weight within a half day.

Closures saturated with sulfur dioxide were immersed in water and water sorption was determined in the usual manner. A typical sorption curve is shown in Fig. 4. Since the closures lost weight upon immersion, the weight increase was determined using the initial weights of the closures prior to exposure to sulfur dioxide. As can be seen from Fig. 4, a break was observed in the curve. The sorption values represented by the first portion of the curve were composed of residual sulfur dioxide and sorbed water, the latter portion being composed either of water alone or of water and a constant amount of sulfur dioxide. Extrapolation of the latter portion of the curves shows that the sorption is greatly increased by the presence of sulfur dioxide in the rubber.

The abnormality of the curves obtained with sodium bisulfite can be explained on the basis of these experiments. If curves obtained with various concentrations of sulfur dioxide are superimposed upon the curve obtained with sodium bisulfite it can be seen that the upward shift in the bisulfite curve is due to the increasing concentrations of sulfur dioxide from the breakdown of sodium bisulfite (Fig. 5).

Potassium bisulfite solution was found to have a constant vapor pressure (34.5 mm.) indicating that equilibrium was attained rapidly and thus no change occurred in sulfur dioxide concentration during the sorption experiments. This explains why sorption from potassium bisulfite solution, though high, followed the normal equation while that from sodium bisulfite exhibited an upward shift.

The results of these experiments indicate that sulfur dioxide is the cause of the high sorption from bisulfite solutions. The finding that sulfur dioxide is sorbed by closures and that it is rapidly desorbed in the presence of water should make it possible to treat closures in such a manner as to maintain an equilibrium between closure and solution, thus keeping the sulfur dioxide or bisulfite in the solution to act as a preservative. This might best be done by allowing an excessive amount of sulfur dioxide gas to be sorbed on the closures and then placing the closures in a solution of bisulfite of the concentration to be required in the product. Desorption would be expected to take place rapidly to reach equilibrium, and the closures would be ready for use.

Effects of Special Treatments on Sorption of Closures.—It is a common practice in industry to give rubber closures for injection special treatments before using them. The choice of treatment depends on the closure type as well as the solution to be packaged, and these procedures are reported to reduce the incidence of incompatibility. This leads one to believe that the process changes the closures in some way so as to make them less reactive, perhaps by leaching of soluble materials from the compositions. It was thought that if an appreciable amount of leaching does take place during the treatment process this may be indicated by sorption measurement, and that sorption measurements might thus serve to evaluate treatment procedures. If these treatments do not leach materials from within the closures but simply remove surface particles, sorption would not be affected and would not be useful for evaluating the treatments.

To test the possiblity of using sorption measurements to measure the effect of treatments on closures, three treatment procedures were selected which have been reported to enhance stability of certain closures (8). The treatments were as follows:

(*a*) Pyrophosphate treatment. Gum 79 and Pink 124 closures were agitated in hot 1% sodium tetrapyrophosphate for thirty minutes, in hot tap water for fifteen minutes, in hot distilled water for fifteen minutes, and then autoclaved in distilled water for thirty minutes.

(*b*) Sulfuric acid treatment. Gum 79 closures were placed in a flask, covered with tap water and autoclaved at fifteen pounds for fifteen minutes. The autoclaving was repeated twice with fresh portions of tap water. The closures were then covered with 2% sulfuric acid and autoclaved for fifteen minutes. They were then agitated in hot tap water fifteen minutes and hot distilled water fifteen minutes. They were finally given a thirty-minute autoclaving in contact with distilled water.

(*c*) Sodium bisulfite treatment. Gum 79 closures were treated as in procedure 2 through the autoclaving with sulfuric acid. At this point the closures were soaked for seventy-two hours in 0.2% sodium bisulfite solution. They were then agitated for fifteen minutes in hot tap water, fifteen minutes in hot distilled water, and finally given a fifteen-minute autoclaving in contact with distilled water.

Three closures selected at random from each of the batches given these treatments were dried to constant weight and their sorption from distilled water was determined in the usual manner. Results of sorption tests on the washed closures, expressed as extrapolation values, are shown in Table II, and the logarithmic plots are shown in Fig. 6 and Fig. 7.

TABLE II.—RESULTS OF SPECIAL TREATMENTS OF CLOSURES

Rubber Composition	One-Year Extrapolation Values, Gm. × 10^4			
	Special Treatments			
	None	Method 1	Method 2	Method 3
Gum 79	283	389	402	450
Pink 124	495	603	...	...

In all cases there was an increase in sorption due to the treatments. As can be seen from Fig. 6, Gum 79 closures treated with sodium tetrapyrophosphate (method 1) and sulfuric acid (method 2) exhibited identical sorption curves with higher intercepts (log *k*) than obtained with untreated closures, but with the same slope (*n*) as the untreated.

Taylor and Kemp (6) reported that soft rubber sorbed water at a higher rate than did hard, the sorption curves of the two differing only in intercept. Since autoclaving is known to soften rubber closures, sometimes to the point of tackiness, the increased sorption rate obtained after treatment may

be due entirely to a softening of the rubber composition. The fact that method 1 and method 2 differed in time of autoclaving (thirty minutes and ninety minutes, respectively) would cause one to discard this explanation unless autoclave temperature is the determining factor rather than time of autoclaving. This has not been established as yet, but it is known that a short period of autoclaving will soften closures while longer periods seem not to carry this softening beyond a limit. Whether this limit is reached within thirty minutes is not known.

If it is assumed on the basis of the limited evidence available that softening of rubber causes a change in intercept but not slope of the sorption curve then it would appear that Pink 124 owes its increased sorption to some other factor. As may be seen from Fig. 7 the sorption curve of Pink 124 after treatment 1 shows an increase in slope and a decrease in intercept. It is possible that Pink 124 closures sorbed sodium tetrapyrophosphate during the treatment. If that had occurred it would be expected to show an increased slope in the sorption curve, since this is the type of effect shown by increased amounts of soluble material within the closure. It has been reported (6) that having been penetrated once by water and then dried, rubber will sorb at a higher rate upon re-immersion. This phenomenon has been explained as a deformation or "set" and may be likened to opening channels within the rubber which allow a more rapid penetration. This effect could also have caused the increase in sorption rate noted following the treatments.

It is seen from Fig. 6 that the curve obtained after treatment of Gum 79 closures by method 3 exhibits an abrupt change in slope. It was previously found that bisulfite solutions caused a much higher degree of sorption than with most solutions and thus would cause a higher degree of "set" during treatment which would account for the higher rate shown in the first portion of the curve.

From the results obtained it is apparent that the changes occurring with pretreatment are complex and cannot be evaluated by simple measurement of sorption.

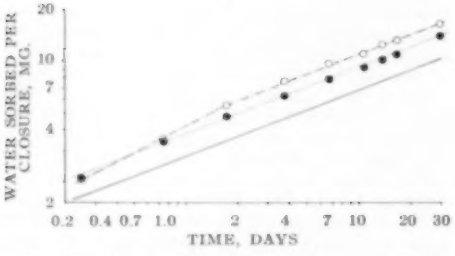

Fig. 6.—Effects of Special Treatments on Sorption of Gum 79 Closures. —○—○— Bisulfite, ...●...●... Pyrophosphate-Sulfuric Acid, —— Untreated.

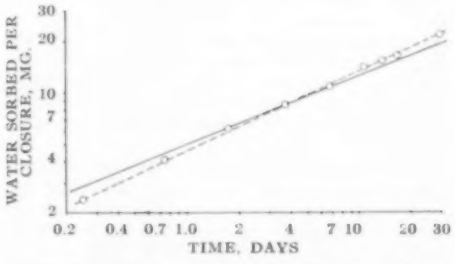

Fig. 7.—Effects of Tetrapyrophosphate Treatment on Sorption of Pink 124 Closures. ——○—— Treated, —— Untreated.

SUMMARY

1. A logarithmic relationship between sorption of water by rubber closures and depression of vapor pressure due to inorganic salts was derived.

2. An apparatus suitable for vapor pressure measurements of solutions is described.

3. Sodium and potassium chlorides and phosphates were found to affect sorption of closures beyond the extent predicted from vapor pressure considerations.

4. Abnormally high sorption of closures from bisulfite solutions was explained as a result of sorption of sulfur dioxide which enhances the sorption of water by the closures. The continually increasing curve obtained for sorption from sodium bisulfite solutions was shown to be due to the continuing breakdown of the solutions with the liberation of sulfur dioxide. Sulfur dioxide alone was shown to be sorbed by rubber but to be leached readily from the closures when they are placed in water.

5. An attempt was made to evaluate by means of sorption measurements the effects of special treatments commonly used industrially to enhance the stability of rubber closures. Sorption could not be correlated with these treatments.

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Sorption of Water by Rubber Closures for Injections III.*

Effect of Organic Compounds

By GEORGE MILOSOVICH† and ALBERT M. MATTOCKS

The effect of organic compounds on the sorption from solution by rubber closures has been studied. The sorption constants are reported for six rubber closure compositions immersed in twenty-eight solutions of organic compounds. Evidence is presented that vapor pressure, solubility, and sorption by the fillers do not entirely govern the extent of sorption. An explanation based on elasticity is advanced for the effects of organic and inorganic compounds on the sorption of the various rubber compositions.

IN PREVIOUS COMMUNICATIONS the effects of inorganic compounds and washing procedures on the sorption of water by rubber closures from aqueous solutions were reported (1, 2). In this paper the results of an investigation of the effects of organic compounds on the sorption process are presented.

It has been shown (3–7) that rubber closures will sorb certain organic compounds from their aqueous solutions. Such sorption of components may lead to a reduction in potency or to removal of the preservative. Either can be considered to constitute an incompatibility. A further disadvantage may arise if the material sorbed by the closures reacts with the components of the rubber composition to cause discoloration or precipitate formation.

It was believed that a study of the sorption of closures from solutions of organic compounds would be of value to indicate the rate of sorption and the types of compounds sorbed; thus the method might serve to predict incompatibility between closures and solutions. Previous work indicated a relationship between vapor pressure depression and sorption from solutions of inorganic compounds, and it was desired to learn whether a similar relationship exists with organic compounds. Sorption of liquid organic compounds, in the absence of water, was also investigated to learn whether solubilities of compounds in rubber might be used to predict their sorption from aqueous solution.

EXPERIMENTAL

Materials.—Twenty-eight compounds were chosen to represent many of the structural groups found in compounds used in injections. These compounds were reagent or pharmaceutical grade and their solutions (1% unless otherwise stated) were made with triple distilled water. The closures were of the same compositions as used in previous studies: West Company formulations Gum 79, Pink 124, Red 123, Red 87, Black 210, and White 86.

Procedure.—The sorption procedure was the same as that previously reported (1), the tests being carried out at 30.1° for twenty days. Vapor pressures were determined by the apparatus and method previously reported (2).

Results.—Sorption, in most of the cases reported, followed the process described by the equation of Taylor and Kemp (8), $w = kt^n$, where w is the weight gained in time, t, and n and k are constants. The results are reported as the constants (obtained by a least squares fit of the logarithmic plots) in Table I and as the one-year extrapolated values in Table II.

As can be seen from Table II, sorption from most of the solutions was greater than from water alone, although several of the compounds caused a depression in sorption. An analysis of variance of the results (expressed as increased sorption, obtained by subtraction of water values from solution values), Table III, showed that compounds and rubber composition were significant. A breakdown of compound effect showed that it is mainly due to the high sorption obtained from propionic, acetic, and phthalic acid solutions and a breakdown of the rubber composition effect showed that it is due chiefly to the high sorption exhibited by Pink 124.

From the analysis it is seen that a high residual, or error term, was obtained. The residual in this case includes the first order interaction or the variance due to the solutions affecting the closures in different order. For example, Black 210 sorbed *m*-cresol to a greater extent than did the other closures, but with other solutions the sorption of Black 210 was relatively low. Thus, the factors proved significant are only those which have an overall effect significantly greater than these individual combination peculiarities.

Vapor pressure measurements, presented in Table IV, showed no correlation with sorption since many solutions had vapor pressures greater than pure water due to the volatility of the organic compounds. It was thus not possible to obtain the depression of vapor pressure of water caused by introduction of

* Received October 24, 1956, from the College of Pharmacy, University of Michigan, Ann Arbor.
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TABLE I.—SORPTION CONSTANTS FOR RUBBER CLOSURES IN ORGANIC SOLUTIONS

$\log w = n \log t + \log k$

Solution	Closure Type: Gum 79		Pink 124		Red 123		Black 210		White 86		Red 87	
	n	$\log k$	n	$\log k$	n	$\log k$	n	$\log k$	n	$\log k$	n	$\log k$
H_2O	0.390	0.454	0.388	0.699	0.426	0.459	0.370	0.491	0.448	0.355	0.441	0.397
n-Butanol	0.278	0.863	0.340	0.942	0.397	0.716	0.331	0.870	0.410	0.634	0.400	0.649
sec-Butanol	0.358	0.743	0.342	0.931	0.403	0.702	0.338	0.810	0.421	0.581	0.408	0.588
t-Butanol	0.326	0.660	0.352	0.775	0.415	0.591	0.343	0.694	0.416	0.412	0.411	0.471
Benzamide	0.361	0.683	0.330	0.883	0.412	0.616	0.377	0.794	0.470	0.430	0.413	0.489
Benzaldehyde[a]	...	...	0.597	1.317	...	...	...	...	0.510	1.217	...	...
Acetamide	0.356	0.567	0.308	0.822	0.417	0.509	0.336	0.632	0.446	0.408	0.424	0.432
Pyridine	0.304	0.810	0.387	0.827	0.323	0.732	0.221	0.934	0.342	0.648	0.300	0.669
Diethylamine	0.414	0.763	0.555	0.898	0.428	0.767	0.402	0.689	0.468	0.653	0.470	0.688
Triethylamine	0.220	1.148	0.413	1.174	0.292	1.112	0.395	0.886	0.294	1.066	0.320	1.063
Triethylamine methoiodide	0.375	0.463	0.379	0.549	0.392	0.414	0.314	0.567	0.452	0.292	0.427	0.328
Aniline	0.291	0.938	0.293	0.929	0.279	0.851	...	...	0.229	0.821	0.345	0.557
Diethylaniline	0.393	0.506	0.469	0.640	0.496	0.372	0.445	0.553	0.482	0.269	0.502	0.099
Diethylaniline methoiodide	0.417	0.468	0.373	0.614	0.439	0.404	0.341	0.580	0.437	0.348	0.430	0.370
m-Nitroaniline[a]	0.396	0.476	0.492	0.663	0.518	0.352	0.419	0.240	0.452	0.291	0.726	−0.084
Ethyl acetate	0.198	0.936	0.388	0.945	0.237	0.908	0.159	1.093	0.231	0.838	0.224	0.876
m-Cresol	0.199	1.142	0.309	1.028	0.265	1.013	...	...	0.234	1.001	0.236	1.018
Phenol	0.378	0.758	0.386	0.774	0.414	0.661	...	...	0.423	0.625	...	...
Resorcinol	0.400	0.517	0.356	0.717	0.436	0.460	0.344	0.771	0.466	0.381	0.412	0.429
2-Butanone	0.301	0.706	0.322	0.790	0.345	0.630	0.259	0.872	0.352	0.576	0.338	0.626
Acetone	0.356	0.576	0.364	0.676	0.396	0.471	0.301	0.661	0.444	0.392	0.393	0.467
Merthiolate	0.472	0.526	...	...	0.487	0.496	0.375	0.624	0.548	0.386	0.537	0.446
Thioacetic acid	0.248	1.008	0.343	1.161	0.241	1.051	0.293	1.181	0.326	0.925	0.342	0.997
Acetic acid	0.422	0.664	0.653	1.499	0.625	1.151	0.534	1.143	0.699	0.988	0.657	1.290
Oxalic acid	0.378	0.503	0.342	0.650	0.440	0.480	0.401	0.578	0.438	0.387	0.556	0.175
Propionic acid	0.420	0.802	0.689	1.554	0.496	1.340	0.535	1.165	0.665	1.101	0.589	1.465
Citric acid	0.388	0.511	0.332	0.686	0.440	0.398	0.329	0.610	0.438	0.356	0.471	0.198
Benzoic acid[a]	0.357	0.630	0.430	0.777	0.484	0.473	0.428	0.665	0.459	0.453	0.417	0.517
Phthalic acid[a]	0.423	0.574	0.705	1.094	0.691	0.705	0.564	0.810	0.855	0.632	0.788	0.440

[a] Saturated solution.

TABLE II.—ONE-YEAR EXTRAPOLATION VALUES GM. × 10^4 SORBED BY INDIVIDUAL CLOSURES

Solution	Closure Type: Gum 79	Pink 124	Red 123	Black 210	White 86	Red 87
H_2O	283	495	356	275	318	337
n-Butanol	377	604	541	522	483	472
sec-Butanol	457	641	543	474	457	313
t-Butanol	313	475	451	374	301	334
Benzamide	406	535	470	575	431	352
Benzaldehyde	...	7030	...	...	3340	...
Acetamide	301	408	378	311	356	330
Pyridine	388	658	363	316	334	274
Diethylamine	667	2090	731	524	711	780
Triethylamine	515	1700	725	791	659	764
Triethylamine methoiodide	265	331	262	235	282	264
Aniline	483	479	368	...	256	276
Diethylaniline	326	695	440	493	319	346
Diethylaniline methoiodide	344	372	338	284	294	296
m-Nitroaniline	310	839	478	650	281	597
Ethyl acetate	277	869	712	316	269	282
m-Cresol	449	676	492	2130	399	420
Phenol	532	579	526	...	512	...
Resorcinol	348	426	378	449	376	306
2-Butanone	300	412	327	344	301	310
Acetone	308	406	306	270	339	298
Merthiolate	543	...	555	385	617	664
Thioacetic acid	440	1100	466	817	575	747
Acetic acid	556	14860	5650	3245	6010	9400
Oxalic acid	296	336	405	403	323	397
Propionic acid	755	20850	4080	3440	6380	9420
Citric acid	320	344	335	284	301	254
Benzoic acid	351	757	516	578	426	385
Phthalic acid	455	7943	2985	1800	6650	2880

TABLE III.—EFFECTS OF SOLUTIONS OF ORGANIC COMPOUNDS ON SORPTION
TWO-WAY ANALYSIS OF VARIANCE

Source	S. S.	D. f.	M. S.	F
Compound	520,963,252	23	22,650,576	6.93[a]
Propionic acid *vs.* others	248,104,561	1	...	76[a]
Acetic acid *vs.* others	206,234,257	1	...	63[a]
Phthalic acid *vs.* others	57,690,913	1	...	18[a]
Rubber composition	45,531,303	5	9,106,261	2.79[a]
Pink 124 *vs.* others	36,085,068	1	...	11[a].
Residual	375,745,560	115	3,267,353	
Total		143		

[a] Significant at 5 per cent or lower level.

the compounds. The results do indicate, however, that vapor pressure of the organic compound is not the controlling factor of the sorption process. For example, a relatively nonvolatile substance such as phthalic acid caused a very high sorption whereas volatile compounds such as acetone had very little effect. It was thought, therefore, that the controlling factor might be the solubility of the organic compounds in the rubber compositions, and this was determined by measuring the sorption of the closures when immersed in the organic compounds of this group which are liquids. Sorption values obtained in this manner did not follow the equation applied to aqueous solutions, but the weight gain followed four different and rather distinct patterns. An example of each of the four types is shown in Fig. 1, and the closure-compound combinations, classified according to these types, are reported in Table V. An examination of these results showed little correlation between the sorption of pure compounds and sorption from their aqueous solution. Acetic acid, for example, was sorbed to a lesser extent than most organic compounds whereas sorption from its aqueous solution was extremely high. It appears, therefore, that acetic acid (and other organic compounds causing high sorption in aqueous solution) enhance the sorption of water by the closure. More work is required to clarify this effect.

DISCUSSION

From these results and from those reported with solutions of inorganic compounds (1) there appears to be a correlation of the effects on sorption ex-

TABLE IV.—VAPOR PRESSURE OF ORGANIC SOLUTIONS 30.1°

Solution 1 Per cent	V. P., mm.
Triethylamine	41.3
2-Butanone	37.8
Acetone	36.3
Ethyl acetate	36.0
Diethylamine	34.0
sec-Butanol	33.3
Pyridine	33.2
t-Butanol	33.1
n-Butanol	32.9
Phenol	31.9
Aniline	31.9
Resorcinol	31.9
Acetamide	31.8
m-Cresol	31.7
Diethylaniline methoiodide	31.7
Benzamide	31.6
Triethylamine methoiodide	31.6
Oxalic acid	31.6
Diethylaniline	31.4
Propionic acid	31.4
Thioacetic acid	31.3
Merthiolate	31.3
Acetic acid	31.2
Citric acid	30.2
Saturated	
Benzaldehyde	32.9
m-Nitroaniline	31.8
Phthalic acid	31.7
Benzoic acid	31.6
Water	32.0

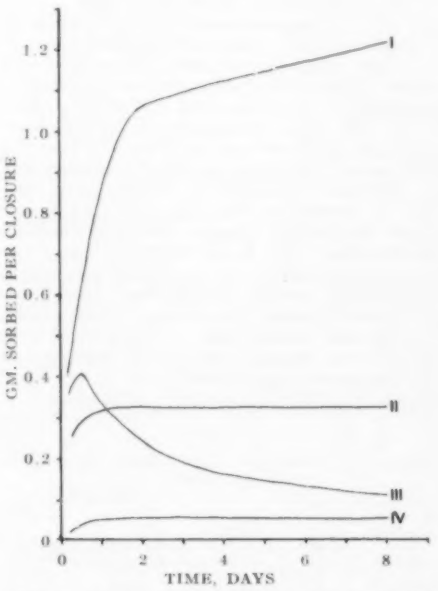

Fig. 1.—Four types of sorption of organic liquids: I—Diethylaniline-pink 124, II—Propionic acid-black 210, III—pyridine-gum 79, IV—acetic acid-gum 79.

TABLE V.—SORPTION OF ORGANIC COMPOUNDS CLASSIFICATION OF CLOSURE-COMPOUND COMBINATIONS

Compound	Closure Composition					
	Gum 79	Pink 124	Red 123	White 86	Black 210	Red 87
Pyridine	III	III	III	III	III	III
2-Butanone	III	III	III	III	III	III
n-Butanol	IV	IV	IV	IV	III	IV
sec-Butanol	IV	IV	IV	IV	III	IV
t-Butanol	IV	IV	IV	IV	III	IV
Benzaldehyde	II	II	III	III	II	III
Ethyl acetate	III	III	III	III	III	III
Acetone	III	III	III	III	II	III
Diethyl aniline	I	I	I	I	I	I
m-Cresol	IV	IV	IV	IV	I	IV
Aniline	IV	IV	IV	IV	I	IV
Acetic acid	IV	IV	IV	IV	II	IV
Propionic acid	II	II	II	II	II	II
Triethylamine	I	I	I	I	IV	I
Diethylamine	I	I	I	I	IV	I

TABLE VI.—SUMMARY OF EFFECTS ON RUBBER COMPOSITIONS

Rubber Composition	Rubber, %	Water Sorption[a]	Inorganic Depression[b]	Sorption Organic Compounds[c]	Sorption Sulfur Dioxide[d]
Pink 124	42	495	340	2430	3206
Red 87	56	337	210	1259	1377
Red 123	61	356	197	932	1270
White 86	63	318	186	1136	1285
Gum 79	96	283	111	397	373

[a] One-year extrapolated values, Gm. × 10^4. [b] Average of extrapolated values for water minus those for 18 solutions of inorganic salts. [c] Average extrapolated values for 24 solutions of organic compounds. [d] Extrapolated values for sorption following sulfur dioxide treatment.

erted by different compounds and the percentage of rubber in the closures, as shown in Table VI. In the case of organic compounds the sorption increased as the percentage of rubber in the closures decreased. If it were assumed that increased sorption with organic compounds was due partly to solution of the compounds in the rubber and that vapor pressure is the sole factor determining sorption of water [suggested by Lowry and Kohman (9)] then one would expect the greatest sorption by closures having the highest percentage of rubber. Since, however, the opposite is true, some other factor must control the sorption process. It was first thought that the filler might be the controlling factor, but sorption measurements on the fillers alone showed no correlation between their tendency to sorb and the sorption of rubber compositions containing them. This has been substantiated by the work of Wing (10) who reported that varying most fillers in both quantity and type had little effect on the sorption of phenol and chlorocresol by rubber compositions.

TABLE VII.—PHYSICAL PROPERTIES OF RUBBER COMPOSITIONS

Rubber Composition	Rubber, %	Hardness (Duro-Shore)	Compression Recovery, %	Specific Gravity
Pink 124	42	50	25	1.67
Red 87	56	42	60	1.27
Red 123	61	38	65	1.27
White 86	63	40	60	1.25
Gum 79	96	35	76	0.90

This leads one to search for other factors which might control the sorption process. A comparison of the known physical properties of the rubber compositions tested is shown in Table VII.

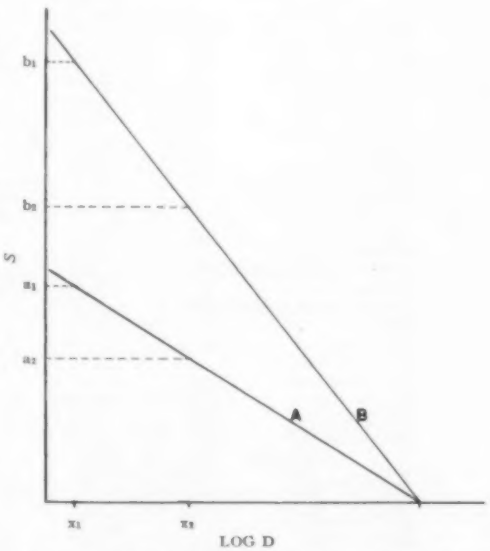

Fig. 2.—Hypothetical plot of sorption, *S*, versus log of vapor pressure depression, log D.

From this table it is seen that specific gravity and hardness increase as percentage of rubber decreases. This is to be expected, since the specific gravity of fillers is greater than that of rubber, and one of the major purposes for adding fillers is to increase hardness. It is difficult, however, to relate hardness or specific gravity directly to sorption. On the other hand, the per cent compression recovery increases as per cent of rubber increases, and this may be the significant factor.

The per cent compression recovery is a measure of elasticity, and the less the elasticity the greater the permanent change which may be expected with a composition when any treatment extends it. Thus a composition with low elasticity would be expected to sorb to a greater extent than one of higher elasticity since the forces retarding sorption are less. The fact that compositions with low elasticity have their sorption affected the greatest with a given reduction of vapor pressure may be explained by the following illustration, Fig. 2. Figure 2 is a hypothetical semilogarithmic plot of water sorption *versus* vapor pressure depression for two compositions, A-high elasticity and B-low elasticity. At a given vapor pressure reduction, x_1, A will sorb A_1 and B will sorb B_1. An increase in vapor pressure depression to x_2 would cause a drop in sorption from B_1 to B_2 and from A_1 to A_2. As seen from the figure, the change is greater for composition B of low elasticity. This effect is shown under the heading Inorganic Depression in Table VI.

Until more results are available this explanation must be considered speculative, but it does point out a possible avenue for preparation of closures which exhibit less sorption as well as to explain the results obtained thus far. Further work is planned to test this hypothesis.

SUMMARY

1. Sorption constants are reported for six rubber closure compositions immersed in solutions of twenty-eight organic compounds.

2. Evidence is presented to show that sorption is not dependent upon vapor pressure of the organic compound.

3. The solubility of the organic compound in rubber is shown not to be the chief factor determining the extent of sorption.

4. Sorption by the fillers was found not to be the primary factor determining the extent of sorption.

5. An explanation based on elasticity is advanced for the effects of organic and inorganic compounds on the sorption of various rubber compositions.

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The Preparation and Pharmacological Properties of Some Chloral Derivatives*

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Two acetals of chloral and several derivatives of trichlorethanol have been prepared and subjected to pharmacological evaluation for CNS-depressant activity. None of them was found to be superior to chloral.

ALTHOUGH chloral hydrate has been long used as an effective central nervous system (CNS) depressant of the hypnotic type it does suffer from the disadvantages of sometimes causing gastric irritation and of producing a recurrent unpleasant after-taste. Many attempts have been made to retain the useful properties of chloral with amelioration of the side effects through reaction of the molecule with other substances, usually hydroxylic. Most of the older attempts at this have been dealt with in the several books written on medicinal chemistry and pharmacology during the last few years and it will suffice here to mention a few of the more recently described substances. Among these are the dioxolanes prepared from glyceric acid (1),

* Received January 20, 1956, from the Warner-Chilcott Research Laboratories, Morris Plains, N. J.

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glycerol esters (2), mephenesin (3), glycuronolactone (4), and certain α-glycols and amino-substituted α-glycols (5); dioxanes derived from 1,3-glycols (5, 6); the hemiacetals with cyclic alcohols (7); the addition products with amides (8–10); and the esters of trichlorethanol with certain dibasic acids (11).

In further efforts to accomplish the above-stated purpose we prepared, by a procedure similar to that described (12) for the preparation of a compound derived from chloral and Bromural, the compound formed by interaction of chloral with carbromal and learned to our

$$Cl_3CCHO + (C_2H_5)_2CCONHCONH_2 \rightarrow Cl_3CCH(OH)NHCONHCOC(Br)(C_2H_5)_2$$

surprise that interaction of the two active moieties destroyed depressant activity in the product. The oral LD_{50} in mice was greater than 2 Gm./Kg. This dose caused tremors, incoordination, running, increased muscle tonus, and impairment of the righting reflexes, but no hypnosis; one out of eight mice died. There apparently was extremely limited absorption from the gastrointestinal tract. Five hundred mg./Kg. orally in mice did not protect against supramaximal electroshock.

Despite the not too promising outlook for activity in the products we did prepare a couple of additional hemiacetals of chloral. The first of these, the bis-hemiacetal made from propylene glycol, had an LD_{50} orally in mice of about 1,500

$$2Cl_3CCHO + HOCH_2CH(CH_3)OH \rightarrow Cl_3CHOCH_2CH(CH_3)OCHCCl_3 \quad (OH, OH)$$

mg./Kg. and an oral HD_{50} (dose level at which 50 per cent of the animals slept) in mice of about 900 mg./Kg.; sleep appeared within ten to fifteen minutes and lasted from three to sixteen hours. The intravenous LD_{50} in mice was over 500 mg./Kg. and the intravenous HD_{50} was 225 mg./Kg.; intraperitoneally the HD_{50} was about 400 mg./Kg. One hundred mg./Kg. intraperitoneally potentiated the hypnotic effect of Evipal® 1.8 times. Neither 100 mg./Kg. subcutaneously nor 1,000 mg./Kg. orally had any analgetic effect.

The other hemiacetal, prepared from 2,2-diethyl-1,3-propanediol which has enjoyed some clinical use, was somewhat different in its

$$2Cl_3CCHO + HOCH_2C(C_2H_5)_2CH_2OH \rightarrow Cl_3CHOCH_2C(C_2H_5)_2CH_2OCHCCl_3 \quad (OH, OH)$$

pharmacological action. The oral LD_{50} in mice was about 1,800 mg./Kg. while the oral HD_{50} was about 1,200 mg./Kg. with light hypnosis coming on one to three hours after the HD_{50} dose and then lasting for five to eight hours. The intraperitoneal LD_{50} in mice was 1,050 mg./Kg. and the intraperitoneal HD_{50} was 615 mg./Kg.; at one-fourth of the oral HD_{50}, which level was only 16 per cent of the oral LD_{50}, the compound potentiated the action of Evipal 3.1 times and the action of chloral hydrate 6.8 times. Two hundred mg./Kg. intraperitoneally produced no analgetic effect. The ED_{50} (dose level at which 50 per cent of the animals were protected) against supramaximal electroshock was 330 mg./Kg. orally (about 18 per cent of the LD_{50}) with the peak effect at about $1^1/_2$ hours. The same dosage intraperitoneally protected nine out of ten mice against Metrazol® convulsions.

NEW APPROACH

We then turned down a different avenue of approach to what was hoped to be a nonbarbiturate drug possessing the useful properties of chloral, basing this approach on consideration of the metabolic fate of chloral in the mammalian organism. According to classical beliefs, chloral is first reduced to trichlorethanol which then is conjugated with glucuronic acid to form the pharmacologically inactive urochloralic acid found in the urine. Lehmann and Knoefel (13, 14) oppose this view because trichlorethanol had a more intense CNS-depressant activity and was longer acting than the proposed parent compound, chloral. In a detailed pharmacological study they showed that: (*a*) trichlorethanol was more potent than the tribrom derivative, a basal anesthetic which has been used clinically for some years under the name Avertin;® (*b*) the duration of narcosis and postanesthetic depression caused by trichlorethanol was even a little greater than that given by Avertin in tertiary amyl alcohol; (*c*) the analgetic action of trichlorethanol was roughly the same as that of Avertin; (*d*) in large doses both compounds caused a fall in arterial pressure due to depression of the medullary and spinal vasomotor centers; (*e*) cardiac function remained unimpaired; (*f*) excretion of trichlorethanol remained at a higher level in man for a period of six hours and then fell off rapidly; (*g*) tolerance was not rapidly developed; (*h*) the material produced no gastric irritation; (*i*) orally administered to rats, the minimal hyp-

notic, anesthetic, and fatal doses of trichlorethanol were 200, 600, and 1,000 mg./Kg., respectively.

A second pharmacological study was reported by Molitor and Robinson (15). They compared the toxicity of trichlorethanol, Avertin, and Avertin in amylene hydrate in mice, rats, rabbits, and dogs. The toxic dose of trichlorethanol was lower than those of the other two preparations. The effect of trichlorethanol on the circulatory system was similar to that of the tribrom derivative, with the exception of the transient appearance of extrasystoles. Death from overdoses resulted from respiratory failure. Repeated administration of trichlorethanol did not affect the liver as determined by a standard function test, and both gross and microscopic examinations of this organ revealed no pathology. Tests for local irritation following rectal and oral administration of trichlorethanol in rats, rabbits, guinea pigs, and dogs revealed no signs of irritation in dogs and rats, although rabbits occasionally showed irritation similar to that seen after Avertin. Gross and microscopic examinations of the heart, liver, kidney, spleen, and intestinal tract of animals treated during several weeks with trichlorethanol failed to show any significant pathologic changes. In view of the essential lack of toxicity in animals, trichlorethanol was subjected to clinical evaluation both in this country and in England. Hewer and Belfrage (16) reported the death of an elderly woman from ventricular fibrillation, or possibly from vagal inhibition, of the heart during the course of rectal administration of trichlorethanol. This case represented the eighteenth administration of the drug and although the authors admitted that it was difficult to implicate trichlorethanol they nevertheless abandoned further clinical study. In this country, Case (17) published a review of the literature, including some animal experiments carried out in her own laboratories. She found that trichlorethanol was more stable and had a slightly higher effective and toxic dose than Avertin; that in contrast to Molitor's findings transient impairment of hepatic function and slight renal damage were observed; that hypnotic and toxic effects in animals were equal at equivalent doses of trichlorethanol and Avertin, except that the former produced greater change in cardiac rate and rhythm while the latter caused greater depression of the blood pressure; and that electrocardiographic tracings showed no abnormalities following the use of trichlorethanol in dogs with induced myocardial damage. When trichlorethanol was given to patients individual variation in response was considerable, as with Avertin, but a larger dose was required; the hypnotic action was as prompt, but not so lasting. She concluded that trichlorethanol may be used interchangeably with Avertin as a basal anesthetic. Marshall (18) also has recently discussed some of the pharmacological considerations of the chloral-trichlorethanol relationship.

It seemed to us that an even more desirable compound might be prepared that would have the potency of trichlorethanol with an even greater duration of action if a means could be found to prevent conjugation of the molecule with glucuronic acid. The formation of an ether or ester might satisfy these requirements, provided there was no appreciable loss in activity or gain in toxicity. Toward this end, a few such compounds were synthesized.

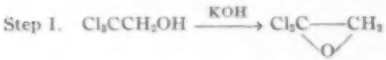
The acetyl derivative of trichlorethanol was originally described by Garzorolli-Thumlackh (19) and its preparation offered no difficulty. Its oral LD_{50} in mice was about 1,000 mg./Kg. and the oral HD_{50} was around 800 mg./Kg. although somewhat irregular, presumably due to lack of constant absorption; the onset of sleep ranged from 2 to 120 minutes with an average onset of about 44 minutes, and the duration of sleep averaged over 200 minutes with the range from 25 to over 300 minutes. Fifty to two hundred mg./Kg. orally did not significantly prolong the sleeping time of animals treated with Evipal; nor did 250 mg./Kg. orally protect mice against supramaximal electroshock. For comparison purposes we obtained some figures of our own on trichlorethanol and found it to have an oral LD_{50} in mice (starved 1 $^1/_2$–3 hours) of 520 mg./Kg. with an HD_{50} orally in mice of 235 mg./Kg., the onset of sleep being within five minutes. Fifty mg./Kg. orally in mice potentiated the sleeping time of Evipal-treated animals 2.5 times, and potentiated the sleeping time of chloral-treated animals 1.6 times, but offered no protection against supramaximal electroshock.

With the thought that the acetyl group might be erratically hydrolyzed and that a more stable ester might be preferable we synthesized the pivalic ester, containing the highly shielding trimethylacetic acid residue. However, our expectation was not realized. The oral LD_{50} of the pivalate in mice was greater than 1,000 mg./Kg. and the rather erratic HD_{50} was greater than 800 mg./Kg. Hypnosis occurred 95 to 300 minutes after treatment (average about 130 minutes). The average duration was more than 200 minutes with a range of hypnosis 10 to 300 minutes. At a dosage of 250 mg./Kg. orally the material did not significantly potentiate the effect of Evipal and at the same dose afforded no protection against supramaximal electroshock.

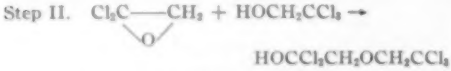
Because we were getting nowhere in improving the effectiveness of trichlorethanol by esterification of its hydroxyl group we decided to study the effect of etherification. Conceivably, the ethers might be very slowly cleaved to the parent material which would exert its effect over a prolonged period. Meerwein and Hinz (20) had prepared the methyl ether of trichlorethanol by treatment of the alcohol with diazomethane. Because the electrical influences in the molecule, as well as the above-mentioned etherification of the hydroxyl group by diazomethane, indicated that the functional group of the molecule is more phenolic than alcoholic we concluded that dimethyl sulfate should afford a convenient means of synthesis of the methyl ether. This proved to be the case, although the product was not very useful. The oral LD_{50} was about 1,500 mg./Kg. and the HD_{50} was no higher. Two hundred and fifty mg./Kg. orally potentiated the effect of Evipal 3.15 times but afforded no protection against supramaximal electroshock. We concluded that the ether linkage was not being cleaved in the animals, possibly through lack of absorption.

We thought that if the molecule were provided with a functional group that would permit its better absorption we might have a useful compound and to this end decided to introduce a carboxyl into the

methyl portion to give the resultant trichlorethoxyacetic acid. It has been reported (19) that treatment of trichlorethanol with potassium hydroxide results in a number of products that includes trichlorethoxyacetic acid, in unspecified yield. In five experiments our yield of the acetic acid derivative ranged from 0% to 3% with the best yield being obtained by addition of small increments of alkali to the alcohol with short (1–2 minutes) heating periods between the additions. The additions were repeated until the solution remained distinctly alkaline after heating, but if heating was continued too long no product at all was obtained. Consideration of the reactants and the product had led us to suggest a mechanism for the course of this very interesting transformation which we believe to be the end product of a sequence of three reactions. In the first step, the alkali dehydrohalogenates the

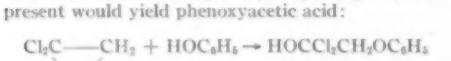


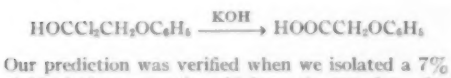
alcohol to asymmetrical dichlorethylene oxide which in the second step is attacked by a second



molecule of the fairly acidic alcohol with opening of the ethylene oxide ring; clearly this step would be inhibited by base. Base is again required for the third step which consists of hydrolysis of the intermediate chloralcohol to the acid. In order to test

the possibility of this hypothesis, we carried out the reaction in the presence of an excess of phenol with the thought that if the supposed dichlorethylene oxide were actually intermediately formed it should also be attacked by the phenol molecule to give a chlorocarbinol which upon hydrolysis by the base present would yield phenoxyacetic acid:





Our prediction was verified when we isolated a 7% yield of phenoxyacetic acid from the reaction mixture. The originally desired trichlorethoxyacetic acid, which had an oral LD_{50} in mice of 750 mg./Kg., was devoid of hypnotic activity and caused delayed deaths in the animals with noticeable hematuria; 250 mg./Kg. did not significantly prolong the sleeping time of Evipal-treated animals and twice this dose afforded no protection against supramaximal electroshock.

As our final compound for study we went in the opposite direction of oxidation and prepared trichloracetamide, a previously known compound that has been described by Zincke and Kegel (21). The oral LD_{50} of this compound in mice was greater than 2 Gm./Kg. and its HD_{50} was about 1 Gm./Kg. Sleep, which lasted an average two hours, came on within two or three minutes and was preceded by incoordination and muscular weakness. Two hundred and fifty mg./Kg. of the compound did not potentiate the action of Evipal and did not protect any animals against supramaximal electroshock. The sodium salt of trichloracetic acid was equally nontoxic and completely devoid of hypnotic activity.

EXPERIMENTAL

N^1 - (2,2,2 - Trichloro - 1 - hydroxyethyl) - N^2- (α - bromo - α - ethylbutyryl) - urea. — A slurry of carbromal (20 Gm.) and freshly distilled chloral (13.6 Gm.) was placed in a pressure bottle and heated on a steam bath. After about 20 minutes the reaction mixture was nearly clear and after 24 hours had become quite viscous and contained some solid. The mixture was then dissolved in hot petroleum ether (b. p. 60–80°) and the solution was permitted to slowly cool with occasional scratching. The product crystallized slowly and the mixture was chilled only after no further recrystallization would occur at room temperature. After several recrystallizations the product melted at 86.5–87°.

Anal.—Calcd. for $C_9H_{14}BrCl_3N_2O_3$: C, 28.11; H, 3.67; total halogen, 36.89; N, 7.29. Found: C, 28.37; H, 3.85; total halogen, 37.17; N, 7.39.

Propylene Glycol bis-(Chloral Hemiacetal).—Freshly distilled ice-cold propylene glycol (7.6 Gm., 0.1 mole) was added portionwise to ice-cold freshly distilled chloral (30 Gm., 0.2 mole) maintained in an ice-bath. The solution was kept in a refrigerator for one month, after which the solid reaction mixture was recrystallized twice from petroleum ether (b. p. 60–80°). The yield of pure product, m. p. 78–80°, was practically quantitative.

Anal.—Calcd. for $C_7H_{10}Cl_6O_4$: C, 22.67; H, 2.72; Cl, 37.37. Found: C, 22.62; H, 2.99; Cl, 37.08.

2,2-Diethyl-1,3-propanediol bis-(Chloral Hemiacetal).—This material was prepared similarly to the propylene glycol derivative described above except that only two days storage in the refrigerator was necessary for complete crystallization. After recrystallization from petroleum ether (b. p. 60–80°) the product melted at 97–99°. It, too, was obtained in practically quantitative yield.

Anal.—Calcd. for $C_{11}H_{18}Cl_6O_4$: C, 30.94; H, 4.25; Cl, 49.84. Found: C, 31.07; H, 4.44; Cl, 49.48.

2,2,2-Trichloroethyl Acetate.—This was a known compound (19) and was prepared by us by the same procedure as described below for the pivalate. Our product was obtained in 54% yield, boiled at 65° (16 mm.), had n_D^{25} 1.4545, and analyzed correctly for carbon and hydrogen.

2,2,2-Trichloroethyl Pivalate.—To a solution of pyridine (25.5 Gm., 0.37 mole) in 75 ml. of toluene there was added dropwise a solution of pivalyl chloride (33.8 Gm., 0.28 mole, b. p. 133°) in 75 ml. of toluene and to the resulting white suspension there was then added dropwise a solution of trichlorethanol (35 Gm., 0.23 mole) in 75 ml. of toluene. The reaction mixture was refluxed for 2.5 hours and then cooled and washed successively with water, 5% sodium carbonate, 4 *N* hydrochloric acid, and water. The dried organic layer was fractionally distilled to give 43 Gm. (79% yield) of product that boiled at 85° (16 mm.) and had n_D^{25} 1.4441.

Anal.—Calcd. for $C_7H_{11}Cl_3O_3$: C, 36.00; H, 4.75. Found: C, 36.18; H, 4.88.

2,2,2-Trichloroethyl Methyl Ether.—This has been prepared by the diazomethane route (20) but we found it much more expedient to use the following procedure. To a mixture of trichlorethanol (15 Gm., 0.1 mole) and dimethylsulfate (15 Gm., 0.2 mole) there was added dropwise with cooling and stirring 42 ml. of 5.8 *M* potassium hydroxide. After one hour the reaction mixture was extracted with ether, the ethereal layer was washed first with dilute hydrochloric acid and then with water and was then fractionately distilled to furnish 10 Gm. (62% yield) of product that boiled at 139–141° and analyzed correctly for carbon and hydrogen.

2,2,2-Trichlorethoxyacetic Acid.—Our preparation of this material is similar to that described in the literature (19). Seventeen ml. of 32.5% potassium hydroxide was added slowly to 10 Gm. (0.067 mole) of trichlorethanol and after the addition was complete the exothermic reaction caused the mixture to boil vigorously. At the end of this spontaneous boiling the mixture was heated on a steam bath for about 15 minutes and then allowed to stand overnight. The following morning the slightly basic reaction mixture was again heated on the steam bath for about 15 minutes and then acidified with dilute sulfuric acid. The oil that separated was extracted with ether. The ether was dried and the solvent removed to leave 0.2 Gm. (2.9% yield) of product that melted at 69° and could be recrystallized from water.

Phenoxyacetic Acid.—To a solution of trichlorethanol (10 Gm., 0.067 mole) and phenol (6.75 Gm., 0.7 mole) there was added dropwise and with vigorous stirring 34 ml. of 32.5% potassium hydroxide. No immediate reaction was observed but after the mixture had been warmed on a steam bath for a few minutes a very vigorous reaction set in and continued for about 20 minutes. After standing overnight and then being heated for an additional few minutes the slightly basic reaction mixture was acidified and extracted with ether. The dried ethereal extract was evaporated to leave an oil which recrystallized on seeding with phenoxyacetic acid. The product weighed 0.78 Gm. (7.5% yield), melted at 97–98°, could be recrystallized from water and did not depress the melting point of authentic phenoxyacetic acid.

Trichloracetamide.—This is a known compound (21) and was prepared from trichloracetyl chloride and ammonia in ether. After recrystallization from water it melted at 140–141°.

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Diisocyanates in the Characterization of Alcohols*

By ANGELO F. SUMMA and PAUL J. JANNKE

Two new isocyanates, diphenylmethane-4,4′-diisocyanate and 2,4-tolylene diisocyanate, were studied with respect to their applicability in the characterization of alcohols. The preparation, purification, and analysis of the urethans are described. Analysis was accomplished by means of a short and convenient nonaqueous titration method.

WURTZ' DISCOVERY (1838) of the basic reaction between ethyl isocyanate and ethanol provided a mechanism which was adapted many years later to the characterization of hydroxyl compounds. The phenyl (1–3) and α-naphthyl (4, 5) urethans became popular derivatives even though some of the former melted a bit too low. The need for higher melting esters of the low molecular weight alcohols and the need for reagents capable of reacting with tertiary alcohols in particular stimulated the introduction of a series of substituted aryl isocyanates which are now commonly employed

* Received Jan. 30, 1957, from the School of Pharmacy, University of Connecticut, Storrs.

in characterization studies.

Although the diisocyanates were described by Snape (6) in 1886, their reactions with alcohols apparently were not studied until 1947 when Bayer (7) established modern polyurethan chemistry. The purpose of this study was to prepare a variety of diurethans from selected alcohols and to determine whether or not these derivatives are suitable for the characterization of the alcohols.

DISCUSSION

A variety of diurethans was prepared from selected primary, secondary, and tertiary alcohols and phenols. The diisocyanates employed were diphenylmethane-4,4′-diisocyanate[1] (97.5–100% pure) and 2,4-tolylene diisocyanate[1] (97–100% pure, containing not more than 1% of the 1,6 isomer).

Reactions involving alcohols of lower molecular weight were immediate, without the application of heat or a catalyst. In some instances, the reactions were exothermic. In the case of the higher molecular weight alcohols and the phenolic compounds, the application of heat and the introduction of pyridine or triethylamine were required to initiate the reaction. No generalizations can be drawn on the reactivity of primary, secondary, and tertiary alcohols with these isocyanates. Cholesterol, linalool, and chlorobutanol did not form diurethans suitable for characterization purposes, whereas menthol and borneol failed with 2,4-tolylene diisocyanate and geraniol did not respond to treatment with diphenylmethane-4,4′-diisocyanate. Interference from moisture was a factor in all cases.

Usually, the urethans are analyzed through the determination of nitrogen by the Kjeldahl method. However, this procedure is inclined to be tedious and so attention was focused upon the possibility of utilizing a simple titration in a nonaqueous medium. According to Fritz (8), compounds having the formula A—NH—A′ can be titrated as acids if A and A′ are any combination of the following groups:

—C—R,	—C—H,	—C—OR	and	—C—NHAr
‖	‖	‖		‖
O	O	O		O
I	II	III		IV

Type III is one of the groups found in the structure of the urethans. The other group is a substituted aryl group in so far as this work is concerned (tolylene and diphenylenemethane, respectively) and in this sense it constitutes a supplement to Fritz's observations.

Moss, *et al.* (9), introduced ethylenediamine as a solvent for the titration of phenols and other weak acids. It not only is an excellent solvent for all types of weak acids, but it also increases their effective acid strength. Potassium methoxide in benzene-methanol was used as titrant and *o*-nitroaniline served as indicator. The color change at the end point (yellow to orange-red) was sharp so long as anhydrous ethylenediamine was used as solvent and moisture was excluded from the titration mixture. The carefully purified α-naphthyl urethans of thymol and carvacrol were used to evaluate the efficiency of the titration procedure. The results were very encouraging and consequently the procedure was applied in the analysis of the diurethans.

In all instances where the reactions between the hydroxyl compounds and the isocyanates were positive, the diurethans were formed with but one exception, namely in the case of isoamyl alcohol and 2,4-tolylene diisocyanate. Repeated experiments invariably yielded the mono-isoamyl urethan.

Although the diurethans were most generally produced, they did not react uniformly in the titration procedure. The ethyl, isopropyl, *n*-butyl and *n*-amyl-2,4-tolylene diurethans functioned as monoprotic acids as is shown by their neutralization equivalents being the same as their respective molecular weights. The ethyl, *n*-propyl, isopropyl, *n*-butyl, *t*-butyl, *n*-amyl, isoamyl and menthyl-diphenylmethane-4,4′-diurethans also functioned as monobasic acids when titrated in a nonaqueous system with potassium methoxide. The remaining diurethans of both isocyanates were diprotic, and consequently their neutralization equivalents approximate one-half of the corresponding molecular weights (Tables I and II).

EXPERIMENTAL

Preparation of Diurethans.—In general, the method employed for the preparation of the diurethans was the standard procedure described by Shriner, Fuson and Curtin (10). In those instances where the reaction was spontaneous, the mixtures were allowed to cool before isolation of the reaction product was attempted. For reactions involving the higher alcohols and phenols, heat was applied by means of a boiling water bath, and a drop or two of pyridine or triethylamine was added to start the reaction (Tables I and II).

In order to prepare the 2,4-tolylene diurethans of geraniol, borneol, menthol, thymol, and carvacrol, equal amounts of the diisocyanate and the hydroxyl compound, together with one drop of pyridine (11), were heated in a loosely stoppered flask on a steam bath for 15 minutes. Crystallization was induced by scratching the sidewalls of the flask and then 10 ml. of dry benzene was added. The mixture was heated to effect solution and filtered. Hexane was added to the filtrate until turbidity resulted whereupon crystallization was allowed to proceed overnight. The crystals were collected by filtration and washed with hexane containing a little benzene.

In those instances (Tables I and II) where the urethans could not be purified directly by recrystallization from boiling ligroin, the reaction products were treated with hot ethylene dichloride (12) and recrystallized from boiling ethanol.

Standardization of Potassium Methoxide Solution.—An approximately 0.1 *N* solution of potassium methoxide in benzene-methanol prepared in the usual manner was standardized at frequent intervals against carefully purified ethyl α-naphthyl urethan by adapting the method of Fritz (8) to suit the purpose. Two drops of *o*-nitroaniline indicator (0.15 Gm. in 100 ml. dry benzene) was added to 15

[1] Diphenylmethane-4,4′-diisocyanate (Mondur M) and 2,4-tolylenediisocyanate (Mondur TDS) were furnished by the Mobay Chemical Co., St. Louis 4, Mo.

TABLE I.—2,4-TOLYLENEDIURETHANS

Derivative	Reaction Conditions: Heat	Reaction Conditions: Catalyst	Recrystallization Solvent	Melting point,[e] °C.	Neutn. Equiv.: Calcd.	Neutn. Equiv.: Found	Mol. Wt.: Theoret.	Mol. Wt.: Calcd.
Methyl-	—	—	Methanol	177	119.12	120.8	238.24	241.6
Ethyl-	—	—	Ethanol	114	266.3	266.6	266.3	266.6
n-Propyl-	—	—	[c]	109	147.17	144.3	294.34	288.6[f]
Isopropyl-	—	—	[c]	118	294.34	294.08	294.34	294.08[f]
n-Butyl-	—	—	[c]	77	322.4	322.9	322.4	322.9[f]
tert-Butyl-	—	—	[d]	208	161.2	162.6	322.4	325.2
n-Amyl-	—	—	[c]	86	350.46	348.3	350.46	348.3[f]
Isoamyl-	—	—	[c]	81	262.31[g]	261.2	262.31[g]	261.2
Cinnamyl-	—	—	[c]	117	221.25	220.3	442.5	440.6
Benzyl-	—	—	[c]	102	195.21	195.03	390.42	390.06
Geraniol-	+	[c]	[d]	89	241.32	241.2	482.64	482.4
Menthol-	+	[c]	[d]	174–179	[h]	[h]	...	...
Borneol-	+	[a]	[d]	217–220	[h]	[h]	...	...
Cholesterol-	+	[a]	[d]	184–187	[h]	[h]	...	...
Linalool-	—	[a]	[d]	228–232	[h]	[h]	...	...
Phenol-	+	[b]	[d]	144	182.2	181.1	364.4	362.2
Chlorobutanol-	+	[b]	[d]	189–194	[h]	[h]	...	...
Thymol-	+	[c]	[d]	190	237.29	236.1	474.58	472.2
Carvacrol-	+	[a]	[d]	159	237.29	237.9	474.58	475.8

[a] Triethylamine. [b] Pyridine. [c] Ligroin and ethanol. [d] Ethylene chloride and ethanol. [e] Melting points have ±0.5–1° C. range except where indicated. [f] Titrated as monoprotic acid. [g] Based on monourethan. [h] Results unsatisfactory.

TABLE II.—DIPHENYLMETHANE-4,4′-DIURETHANS

Derivative	Reaction Conditions: Heat	Reaction Conditions: Catalyst	Recrystallization Solvent	Melting point,[d] °C.	Neutralization Equivalent: Calcd.	Neutralization Equivalent: Found	Mol. Wt.: Theoret.	Mol. Wt.: Calcd.
Methyl-	—	—	Methanol	190	157.53	158.2	315.06	316.4
Ethyl-	—	—	Ethanol	135	343.12	343.4	343.12	343.4[e]
n-Propyl-	+	—	[b]	118	371.16	374.3	371.16	374.3[e]
Isopropyl-	—	—	[b]	153	371.16	372.9	371.16	372.9[e]
n-Butyl-	+	—	[b]	121	399.22	399.0	399.22	399.0[e]
tert-Butyl-	+	—	[c]	275	399.22	400.3	399.22	400.3[e]
n-Amyl-	—	—	[b]	111[f]	427.28	425.5	427.28	425.5[e]
Isoamyl-	—	—	[b]	105	427.28	425.1	427.28	425.1[e]
Cinnamyl-	—	[a]	[c]	169	318.06	316.7	636.13	633.4
Benzyl-	—	—	[c]	197	233.62	233.2	467.24	466.3
Geraniol-	+	[a]	No deriv.	...	...	...	...	...
Menthol-	+	[a]	[c]	172	563.50	560.9	563.50	560.9[e]
Borneol-	+	[a]	[b]	197	279.73	278.6	559.46	557.2
Cholesterol-	+	[a]	[c]	188–193	Results unsatisfactory		...	...
Linalool-	+	[a]	No derivative	...	...	...	...	...
Phenol-	+	[a]	[c]	187	220.61	221.6	441.22	443.2
Chlorobutanol-	+	[a]	[c]	192–196	Results unsatisfactory		...	...
Thymol-	+	[a]	[c]	162	275.7	270.6	551.4	541.2
Carvacrol-	+	[a]	[c]	162	275.7	274.8	551.4	549.6

[a] Pyridine. [b] Ligroin and ethanol. [c] Ethylene chloride and ethanol. [d] Melting points have ±0.5–1° C. range except where indicated. [e] Titrated as monoprotic acid. [f] Decomposed.

to 20 ml. of anhydrous ethylenediamine, and potassium methoxide solution was added dropwise from a microburet until the color change was produced in the indicator. The accurately weighed sample, usually about 40 mg., was then introduced and titration was continued to the end point. The titration mixture was protected from contact with the atmosphere by keeping the flask closed as much as possible. On the basis of the neutralization equivalent calculated for ethyl α-naphthyl urethan (theoretical N.E. 215.17; found, ranging from 216.7 to 219.0) the efficiency of the reaction was established between 100.7 and 101.7%.

Thymol and carvacrol α-naphthyl urethans were also used in this evaluation. Theoretical N.E. 319.38; found 313.0, corresponding to an efficiency of 98.0% for the reaction.

Analysis of the Diurethans.—The diurethans prepared from each of the two isocyanates and the various hydroxyl compounds were analyzed by titration of the respective samples dissolved in anhydrous ethylenediamine with 0.1 *N* potassium methoxide solution, as described under the standardization of the solution. Samples taken ranged from 0.1 to 0.4 millequivalents. The results are given in Tables I and II.

SUMMARY

The use of 2,4-tolylene diisocyanate and diphenylmethane-4,4′-diisocyanate as reagents for the characterization of alcohol was investigated. The reagents were found to react particularly well with the lower molecular weight primary and secondary alcohols.

Analysis of the urethans by titration in nonaqueous solution was accomplished, using anhydrous ethylenediamine as the solvent and potassium methoxide in benzene-methanol as titrant in the presence of *o*-nitroaniline indicator. The method is brief, simple, and accurate.

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Amine Derivatives of Cyanuric Chloride III.* Piperazines and Ethylenediamines

By WILLIAM O. FOYE† and LESTER CHAFETZ‡

A series of basically-substituted *s*-triazines was prepared by condensing cyanuric chloride and 2,4-diamino-6-chloro-*s*-triazine with monoethoxycarbonyl and monoalkyl piperazines and ethylenediamines. Use was made of ultraviolet absorption spectra in characterizing the substituted *s*-triazine salts which gave erratic results on elemental analyses. Several of the *s*-triazines showed *in vitro* but no *in vivo* activity against *Endamoeba histolytica* in rats. One of the compounds produced a significant diuresis when administered orally to dogs.

RELATIVELY few basically-substituted *s*-triazines have been prepared for pharmacological evaluation. A diamine derivative reported in a previous paper (1) of this series, however, 2,4,6-tris-(β-diethylaminoethylamino)-*s*-triazine, showed both amebacidal and *in vivo* antitubercular properties. Cavallito (2) later found that quaternary salts of bis- and tris-dialkylaminoalkylamine derivatives of cyanuric chloride were curarimimetic. A series of cyclicaminoalkylamino-*s*-triazines (3), previously prepared, was reported to have little or no antimalarial or trypanosomicidal activity, however. The preparation of piperazine and ethylenediamine derivatives of the *s*-triazine ring seemed, therefore, worthwhile as a means of providing other *s*-triazines containing basic substituents for evaluation as potential drugs. The necessity of using a diamine rather than a monoamine to provide a basic group becomes apparent on examination of the basic ionization constant of melamine ($K_b = 1 \times 10^{-9}$) (14).

* Received September 24, 1956, from the School of Pharmacy, University of Wisconsin, Madison.
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METHODS OF PREPARATION

The only piperazinyl-*s*-triazine which has been reported is 2,4-diamino-6-(1′-piperazinyl)-*s*-triazine, obtained as a byproduct by Detweiler and Amstutz (4). Good yields of diamine derivatives of *s*-triazine were realized, however, from the condensation of cyanuric chloride with ethyl 1-piperazinecarboxylate and the corresponding ethylenediamine. The mono- and disubstituted triazines were easily synthesized in aqueous medium using Thurston's procedure (5), which permitted the use of the amine hydrochlorides. Trisubstitution of cyanuric chloride by the N-ethoxycarbonyldiamines could not be effected in this manner, probably because of the insolubility of the disubstituted triazines. When the reaction was tried in 1-propanol, however, solvolysis of the chlorine occurred. Analytical results

indicated that the piperazine derivative was converted to a hydroxy compound, 2,4-bis-(4′-ethoxycarbonyl-1′-piperazinyl)-6-hydroxy-*s*-triazine, while the ethylenediamine-substituted triazine apparently reacted with the propanol to form the corresponding 6-propoxy derivative. Some precedent for this type of behavior has been observed in the work of Thurston, *et al.* (6).

Kaiser and co-workers (7) have shown that the reactivity of intermediate 2,4-diamino-6-chloro-*s*-triazines varies with the degree of substitution on the amino nitrogens of the substituting reagent. Higher temperatures were required for condensation with secondary amines than with primary amines. Accordingly, boiling xylene was used as reaction medium for the preparation of 2,4,6-tris-(4′-ethoxycarbonyl-1′-piperazinyl)-*s*-triazine, while the synthesis of the corresponding ethylenediamine derivative was accomplished in refluxing toluene.

Melamine (2,4,6-triamino-*s*-triazine) derivatives of piperazine and ethylenediamine were readily obtainable by condensation of the ethoxycarbonyldiamines with 2,4-diamino-6-chloro-*s*-triazine in aqueous suspension. Monoalkylated piperazines were prepared for condensation with cyanuric chloride or 2,4-diamino-6-chloro-*s*-triazine by hydrogen bromide cleavage of the N-ethoxycarbonyl-N′-alkyldiamines using a method previously described (8). An attempt to condense an N-ethoxycarbonyl-N′-alkyldiamine with 2,4-diamino-6-chloro-*s*-triazine without prior removal of the ethoxycarbonyl group was made with 1-ethoxycarbonyl-4-isopropylpiperazine and was successful. The product obtained had a similar ultraviolet absorption spectrum to its methyl and ethyl homologs and its carbon-hydrogen analysis agreed fairly well with that for the expected compound.

Attempts to cleave the ethoxycarbonyl groups from the N-ethoxycarbonyl-N′-triazinyl piperazines and ethylenediamines by dry hydrogen bromide appeared to be successful in view of the usual liberation of carbon dioxide and ethyl bromide gases. The products, however, did not give correct analyses for carbon, but the hydrogen analyses generally agreed with the theoretical values. A similar situation was encountered in the analysis of 2,4,6-tris-(4′-ethyl-1′-piperazinyl)-*s*-triazine trihydrochloride where incorrect carbon but correct hydrogen values resulted. Difficulties in the elemental analyses of some *s*-triazine derivatives have been noted by others (5, 9). These triazines apparently depolymerized to cyanogen compounds which reacted with the copper oxide in the combustion train. Higher combustion temperatures were sometimes successful in decomposing these salts. In the case of the diamine derivatives of *s*-triazine described below, none of the ethoxycarbonyl-containing compounds were amenable to analysis by the Kjeldahl method, but good results were had with combustion methods for carbon, hydrogen, and nitrogen. On the other hand, satisfactory carbon analyses were not obtained for any of the salts prepared. Support for the expected structures in these cases was obtained through the comparison of ultraviolet absorption spectra with the other members of the series and finding expected absorption maxima as well as consistent molar extinction coefficients. The analytical results obtained for two of the salts prepared are included in Table I.

ABSORPTION SPECTRA

Characteristics of the ultraviolet absorption spectra of the piperazine and ethylenediamine derivatives of *s*-triazine are listed in Table II, along with literature values of related *s*-triazines in Table III. Several correlations may be observed which appear to be valuable in the characterization of *s*-triazines. Relatively small changes occurred in the wavelength of maximum absorption when amino substituents replaced chloro groups, but the molar extinction coefficients increased significantly. The piperazine derivatives absorbed at longer wavelengths than the corresponding ethylenediamines, but an increase in the number of amine substituents in the *s*-triazine ring caused a progressive hypsochromic shift to shorter wavelengths. The bathochromic effect of dialkylamino groups with respect to monoalkyl substituents on the *s*-triazine ring has also been observed by Hirt and Salley (10).

A comparison of the spectra of melamine and ammeline (2,4-diamino-6-hydroxy-*s*-triazine) derivatives shows that while the ammeline derivatives absorb at nearly the same wavelengths as the melamines, their molar extinction coefficients are greater. This intensity of absorption is also greater than that of the corresponding chloro compounds. In the case of melamine and ammeline itself (prepared for this study by hydrolysis of 2,4-diamino-6-chloro-*s*-triazine), the molar extinction coefficient doubled in acid solution when an amino was replaced by a hydroxy group.

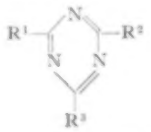
Absorption spectral curves showing maxima near the lower wavelength limit of the quartz ultraviolet region were found to be characteristic of the mono-N-substituted melamines prepared. Similar spectra have been recorded for N-ethylmelamine and N,N-diethylmelamine (10). Hexamethylmelamine, which should show a similar absorption maximum to that of 2,4,6-tris-(4′-ethyl-1′-piperazinyl)*s*-triazine, had a peak at approximately 232 mμ (10), which compares favorably with the peak at 227 mμ found for the tris-piperazinyl derivative. Both the absorption maximum and molar extinction coefficient of 2-(1′-piperazinyl)-4,6-diamino-*s*-triazine hydrobromide were also closely similar to those of the other mono-piperazinyl melamines.

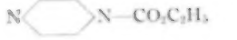
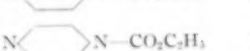
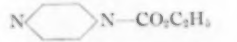
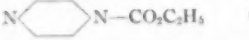
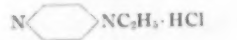
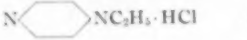
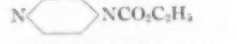
BIOLOGICAL RESULTS[1]

Several of the *s*-triazines were tested against *Endamoeba histolytica* both in the presence and absence of multiplying bacteria. The effective minimum concentrations for inhibition of growth were in the neighborhood of 1:2000 to 1:4000 parts of media. The most favorable activity was shown by 2-(4′-ethoxycarbonylpiperazinyl)-4,6-dichloro-*s*-triazine with an effective minimum concentration of 1:8000 in the presence of mixed bacteria. Consistent effective minimum concentrations of less than 1:2000 were found against *Trichomonas vaginalis* in

[1] The authors are indebted to Drs. K. K. Chen and M. C. McCowen of the Lilly Research Laboratories for these results.

TABLE I.—PIPERAZINE AND ETHYLENEDIAMINE DERIVATIVES OF *s*-TRIAZINE



R^1	R^2	R^3	Recrystn. Solvent	M. p.[a]	Yield, %	Formula	Analysis, %[b] Calcd.	Found
	Cl	Cl	Skelly B	162–163°	<100	$C_{10}H_{12}O_2N_5Cl_2$	C: 39.23 H: 4.28 N: 22.88	40.16 4.62 22.98
		Cl	Toluene	236–238°	94	$C_{17}H_{26}O_4N_7Cl$	C: 47.71 H: 6.12	47.79 6.15
			Ethanol-Water	176–178°	91	$C_{21}H_{33}O_6N_9$	C: 52.44 H: 7.15	52.70 7.20
$NHCH_2CH_2NH-CO_2C_2H_5$	Cl	Cl	Ethyl acetate	171–172°	84	$C_8H_{11}O_2N_5Cl_2$	C: 34.30 H: 3.96	34.72 4.16
$NHCH_2CH_2NH-CO_2C_2H_5$	$NHCH_2CH_2NHCO_2C_2H_5$	Cl	Ethanol	190–192°	<100	$C_{13}H_{22}O_4N_7Cl$	C: 41.54 H: 5.90	41.59 6.07
$NHCH_2CH_2NH-CO_2C_2H_5$	$NHCH_2CH_2NHCO_2C_2H_5$	$NHCH_2CH_2NHCO_2C_2H_5$	Ethyl acetate	152–153°	64	$C_{18}H_{33}O_6N_9$	C: 45.85 H: 7.05	46.16 6.94
		OH	Ethanol-Water	103–105°	<100	$C_{17}H_{27}O_5N_7$	C: 49.86 H: 6.65	49.95 6.94
$NHCH_2CH_2NHCO_2C_2H_5$	$NHCH_2CH_2NHCO_2C_2H_5$	OC_3H_7	Benzene	112–114°	90	$C_{16}H_{29}O_5N_7$	C: 48.18 H: 7.32	47.41 7.42
			2-Propanol-Water	280° (decompn.	40	$C_{21}H_{42}N_9Cl_3$	C: 47.86 H: 8.03	43.09 7.93
	NH_2	NH_2	Ethanol	213°	89	$C_{10}H_{17}O_2N_7$	C: 44.93 H: 6.41	45.36 6.46
$NHCH_2CH_2NHCO_2C_2H_5$	NH_2	NH_2	Water	196–198°	94	$C_8H_{15}O_2N_7$	C: 39.82 H: 6.27	39.70 6.16
$NC_4H_8NC_2H_5$	NH_2	NH_2	Ethyl acetate	193–194°	55	$C_9H_{17}N_7$	C: 48.41 H: 7.68	48.97 7.55
$NC_4H_8NCH_3 \cdot H_2O$	NH_2	NH_2	Water	210–211°	88	$C_8H_{17}ON_7$	C: 42.27 H: 7.54	42.21 7.50
$NC_4H_8NCH(CH_3)_2$	NH_2	NH_2	1-Propanol	195°	32	$C_{10}H_{19}N_7$	C: 50.61 H: 8.07	51.17 8.83
$NC_4H_8NH \cdot HBr$	NH_2	NH_2	Abs. ethanol	255–257°	29	$C_7H_{14}N_7Br$	C: 30.44 H: 5.15	32.23 5.16

[a] The melting points were obtained on a Fisher-Johns block and are uncorrected.
[b] The analyses were carried out by the Clark Microanalytical Laboratory, Urbana, Ill., or by the Weiler and Strauss Microanalytical Laboratory, Oxford, England.

TABLE II.—ULTRAVIOLET ABSORPTION SPECTRA

Substituted *s*-triazine	λmax., mμ[a]	εmax. × 10^{-3}
2-(4′-Ethoxycarbonyl-1′-piperazinyl)-4,6-dichloro	243	24.50
2,4-bis-(4′-Ethoxycarbonyl-1′-piperazinyl)-6-chloro	240	28.45
2,4,6-tris-(4′-Ethoxycarbonyl-1′-piperazinyl)	233	66.87
2,4,6-tris-(4′-Ethyl-1′-piperazinyl)·3HCl	227[b]	58.40
2,4-bis-(4′-Ethoxycarbonyl-1′-piperazinyl)-6-hydroxy	233	36.53
2-(2′-Ethoxycarbonylaminoethylamino)-4,6-dichloro	235	19.81
2,4-bis-(2′-Ethoxycarbonylaminoethylamino)-6-chloro	222	35.48
2,4,6-tris-(2′-Ethoxycarbonylaminoethylamino)	217	52.74
2,4-bis-(2′-Ethoxycarbonylaminoethylamino)-6-propoxy	218	39.24
2-(4′-Ethoxycarbonyl-1′-piperazinyl)-4,6-diamino	211	36.26
2-(2′-Ethoxycarbonylaminoethylamino)-4,6-diamino	208	43.95
2-(4′-Methyl-1′-piperazinyl)-4,6-diamino	211[b]	36.40
2-(4′-Ethyl-1′-piperazinyl)-4,6-diamino	215	29.21
2-(4′-Isopropyl-1′-piperazinyl)-4,6-diamino	214	23.81
2-(1′-Piperazinyl)-4,6-diamino·HBr	206	24.82

[a] The Cary Recording Spectrophotometer was used with 95% ethanol as solvent.
[b] Water was used as solvent.

the absence of bacteria. Four of the *s*-triazines, the 2-ethoxycarbonylpiperazinyl-4,6-dichloro, the 2,4-bis-(2′-ethoxycarbonylaminoethylamino)-6-propoxy, the 2,4-bis-(4′-ethoxycarbonyl-1′-piperazinyl-6-chloro, and the 2,4,6-tris-(4′-ethoxycarbonyl-1′-piperazinyl), were found ineffective in rats infected with *E. histolytica* at doses of 500 mg./Kg. given orally.

Tests for diuretic activity were carried out with several of the *s*-triazines using oral doses of 5 and 10 mg./Kg. in dogs. One of the compounds, the 2,4-bis-(4′-ethoxycarbonyl-1′-piperazinyl)-6-chloro derivative, produced a significant diuresis in seven of fifteen dogs and appeared to be similar in potency to Mictine in dogs.

EXPERIMENTAL

Ethyl 1-Piperazinecarboxylate Hydrochloride.—A modification of the method of Baltzly (12) was used which employed piperazine hexahydrate and absolute ethanol. Yields of 50% of product were obtained, m. p. 155–158°. The free base was obtained by neutralization with aqueous alkali followed by continuous extraction of the product with chloroform, drying, and vacuum distillation; b. p. 124–127° (26 mm.).

2-Ethoxycarbonylaminoethylamine Hydrochloride.—To a solution of 60.1 Gm. (1.0 mole) of ethylenediamine, previously distilled over solid potassium hydroxide, in 500 ml. of 85% ethanol was added dropwise 108.5 Gm. (1.0 mole) of ethyl chloroformate. The mixture was then stirred for thirty minutes, made acid to Congo Red with concentrated hydrochloric acid, stirred for two more hours, and filtered. The precipitate, ethylenediamine hydrochloride, was washed with absolute alcohol, and the filtrate was evaporated to dryness *in vacuo*. The residue was dissolved in water and extracted with ether. The ether-extracted aqueous solution was evaporated to dryness under reduced pressure, and the residue was taken up in a minimum quantity of hot absolute ethanol. After addition of ethyl acetate and dry ether, 58.0 Gm. (34%) of white product was obtained which melted at 110–111°.

Anal.—Calcd. for $C_5H_{13}N_2O_2Cl$: C, 35.61; H, 7.77. Found: C, 35.49; H, 7.75.

1,2-bis-(Ethoxycarbonylamino)ethane.—This was obtained by evaporation of the above ether extract and crystallization from ethanol, m. p. 102–103°; Curtius (13) reported a melting point of 113° for this compound. The yield was 50 Gm. or 25% of theoretical.

Anal.—Calcd. for $C_8H_{16}N_2O_4$: C, 47.04; H, 7.90.

TABLE III.—ULTRAVIOLET ABSORPTION SPECTRA OF RELATED *s*-TRIAZINES

Substituted *s*-triazine	Solvent	λmax., mμ	εmax. × 10^{-3}	Ref.
2,4,6-Trichloro	95% Ethanol	241[a]	2.6	
2-Amino-4,6-dichloro	Water	261, 220	1.96, 15.25	(10)
2,4-Diamino-6-chloro	Cellosolve	256, 210	3.25, 35.0	(10)
2,4-Diamino-6-hydroxy	Hydrochloric acid, pH 3	245[a]	19.41	
2,4,6-Triamino	Water	236	2.5	(11)
	Hydrochloric acid, pH 1	236	9.7	(11)

[a] Determined by the authors.

Found: C, 47.06; H, 7.85.

The free base, 2-ethoxycarbonylaminoethylamine, was obtained by adding saturated potassium carbonate solution to the hydrochloride, extracting with chloroform, and distilling *in vacuo;* b. p. 121° (7 mm.).

Monoethoxycarbonyl and Monoalkyl Piperazine and Ethylenediamine Derivatives of Cyanuric Chloride.—To an ice-cooled solution of 4.8 Gm. (0.025 mole) of ethyl 1-piperazinecarboxylate hydrochloride and 4.2 Gm. (0.05 mole) of sodium bicarbonate in 200 ml. of water was added a solution of 4.6 Gm. (0.025 mole) of cyanuric chloride (recrystallized from carbon tetrachloride) in 50 ml. of acetone. The resulting slurry was stirred at 0° for two hours for the formation of a monopiperazinyl dichloro-*s*-triazine. For the formation of a dipiperazinyl monochloro-*s*-triazine, the mixture, containing two equivalents of ethyl 1-piperazinecarboxylate hydrochloride, was stirred at 40–50°, along with the addition of two more equivalents of sodium bicarbonate solution. The ethylenediamine derivatives were formed in identical fashion.

2,4,6-tris-(Diamino)-*s*-triazines.—A mixture of cyanuric chloride (1.85 Gm., 0.01 mole), ethyl 1-piperazinylcarboxylate (9.5 Gm., 0.06 mole), and 100 ml. of xylene was refluxed for six hours. It was then cooled and extracted with 50 ml. of water, and a solid which precipitated was recrystallized from aqueous ethanol. The xylene filtrate was evaporated to dryness under reduced pressure, and the residue was recrystallized from aqueous ethanol, giving a total yield of 5 Gm. of product melting at 176–178°.

The corresponding ethylenediamine derivative was obtained in the same way, except that toluene was used as solvent, and potassium carbonate was necessary to salt the product out of the aqueous extract.

2,4,6 - tris - (4′ - Ethyl - 1′ - piperazinyl) - *s*-triazine trihydrochloride was also prepared in refluxing toluene using 1-ethylpiperazine. The product precipitated on cooling the reaction mixture, was washed with ether, neutralized, extracted with chloroform, evaporated, and reconverted to the hydrochloride with ethanolic hydrogen chloride. The product was recrystallized from ethanol and then aqueous propanol.

Reaction of Monoethoxycarbonyldiamines with Cyanuric Chloride in 1-Propanol.—A stirred solution of ethyl 1-piperazinecarboxylate hydrochloride (11.7 Gm., 0.06 mole) redistilled triethylamine (6.0 Gm., 0.03 mole), 100 ml. of 1-propanol and 5 ml. of water was cooled to 0° and treated slowly with a solution of 3.7 Gm. (0.02 mole) of cyanuric chloride in 100 ml. of 1-propanol. The mixture was refluxed for three hours, during which time a solution of 6.0 Gm. (0.06 mole) of triethylamine in 50 ml. of 1-propanol was added dropwise. The resulting solution was evaporated to dryness under reduced pressure, and the residue was extracted with ether and 5% hydrochloric acid until no solid remained. The ether extract was washed with water, dried, and evaporated to an oil which was crystallized twice from aqueous ethanol.

In the corresponding reaction with 2-ethoxycarbonylaminoethylamine hydrochloride, six equivalents of diamine were used instead of triethylamine. After evaporation of the reaction mixture, the residue was extracted with water and recrystallized from benzene.

Monoethoxycarbonyl and Monoalkyl Piperazine and Ethylenediamine Derivatives of 2,4-Diamino-6-chloro-*s*-triazine.—To an ice-cold solution of 7.8 Gm. (0.04 mole) of ethyl 1-piperazinecarboxylate hydrochloride and 6.8 Gm. (0.08 mole) of sodium bicarbonate in 200 ml. of water was added 5.8 Gm. (0.04 mole) of 2,4-diamino-6-chloro-*s*-triazine.[1] The stirred suspension was heated at 90–95° or on the steam bath for five to six hours, and the product was filtered, washed with water, dried at 70°, and recrystallized. The ethylenediamine analog was prepared in the same fashion.

When the 1-alkylpiperazine bases were used in this reaction, no sodium bicarbonate was used. The products were isolated after filtering infusible residues from the reaction mixtures and then making the filtrates alkaline. The crystals which precipitated were recrystallized from the appropriate solvent.

2 - (4′ - Isopropyl - 1′ - piperazinyl) - 4,6 - diamino-*s*-triazine.—A mixture of 4.4 Gm. (0.03 mole) of 2,4-diamino-6-chloro-*s*-triazine, 6.0 Gm. (0.03 mole) of 1-ethoxycarbonyl-4-isopropylpiperazine, and 200 ml. of 10% sodium hydroxide solution was stirred at room temperature for one hour and at reflux temperature for an additional hour. After cooling, the mixture was filtered, and the filtrate was made strongly alkaline; the crystals which deposited overnight were isolated and recrystallized from propanol.

2 - (1′ - Piperazinyl) - 4,6 - diamino - *s* - triazine Hydrobromide.—To 50 ml. of a 1 *N* solution of hydrogen bromide in glacial acetic acid, prepared by dilution of 30 to 32% hydrogen bromide in glacial acetic acid (Eastman Organic Chemicals), was added 2.7 Gm. (0.01 mole) of 2-(4′-ethoxycarbonyl-1′ - piperazinyl) - 4,6 - diamino - *s* - triazine. The mixture was warmed on the steam bath for six hours, cooled, and filtered; more product was obtained by adding 200 ml. of dry ether to the filtrate. The combined crops (0.8 Gm.) were recrystallized from absolute ethanol.

[1] A gift of the American Cyanamid Company.

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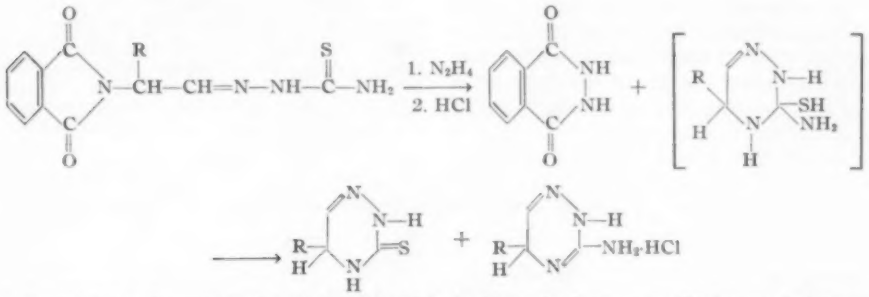
Derivatives of α-Amino Aldehydes III.*

Cyclization Products of Thiosemicarbazones

By WILLIAM O. FOYE† and WINTHROP E. LANGE‡

Removal of the phthaloyl groups from α-phthalimido aldehyde thiosemicarbazones resulted in cyclization to give both 3-thiono- and 3-amino-5-alkyl-2,5-dihydro-*as*-triazines, as well as material of a polymeric nature. Treatment of the amino-2,5-dihydro-*as*-triazines with alkali caused aromatization. No appreciable antiviral activity was found with these compounds.

IN A PREVIOUS PAPER (1) it was shown that hydrazinolysis of α-phthalimidoaldehyde semicarbazones readily removed the phthaloyl groups to give the expected α-aminoaldehyde semicarbazones. When the same method of cleavage was used with α-phthalimidoaldehyde thiosemicarbazones, a cyclization took place and the products were found to be dihydro-*as*-triazines. material was also isolated. Ether extraction of the reaction mixtures produced yellow solids which were soluble in dilute alkali and insoluble in dilute acid. These compounds proved to be thionodihydro-*as*-triazines. Neutralization of the remaining reaction solutions precipitated solids which were identified as amino-*as*-triazines. It was also possible to isolate aminodi-



Equation 1.—Conversion of α-phthalimidoaldehyde thiosemicarbazones to dihydro-*as*-triazines.

This cyclization is not unique, since previous examples of *as*-triazine formation from semicarbazones or thiosemicarbazones are known (2–6).

Treatment of the α-phthalimidoaldehyde thiosemicarbazones with hydrazine as described previously (1) gave the usual quantitative amount of phthalhydrazide after acidification of the reaction solution, but hydrogen sulfide and ammonium chloride were also produced. A red oil which congealed to an elastic polymer-like

hydro-*as*-triazine hydrochlorides by evaporating the reaction solutions to dryness and separating the material from hydrazine hydrochloride by fractional crystallization. Equation 1 illustrates the formation of these compounds.

This procedure was successfully carried out with several α-phthalimidoaldehyde thiosemicarbazones, and the dihydro-*as*-triazines obtained are shown in Table I. The thiono-*as*-triazines derived from glycine and alanine could be obtained only as oils which decomposed on distillation, and the amino-*as*-triazine hydrochloride derived from alanine was extremely hygroscopic. The yields were generally poor.

Evidence for the structures proposed was obtained through carbon-hydrogen analyses, Karl Fischer determinations for water, and both ultraviolet and infrared absorption spectra. The absorption maxima of the thiono-*as*-tria-

* Received November 29, 1956, from the School of Pharmacy, University of Wisconsin, Madison.
Abstracted from the thesis of W. E. Lange submitted to the Graduate School, University of Wisconsin, for the degree of Doctor of Philosophy, August, 1955.
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The authors are indebted to Professor V. W. Meloche for assistance with the infrared absorption spectral determination and to Drs. K. K. Chen and W. S. Boneice of the Lilly Research Laboratories for carrying out the biological determinations.

zines were found to be distinctly different from those of the open chain α-phthalimidoaldehyde thiosemicarbazones, and the amino-*as*-triazines showed maxima corresponding to a typical N—C—N chromophore at 230 and 270 mμ (7). The difference in absorption maximum and large increase in molecular extinction coefficient shown by the free amino-*as*-triazines in contrast to their hydrochlorides is probably due to an aromatization of the former by the action of alkali. This type of behavior has been observed previously (6). The material suspected of being polymeric had an absorption maximum corresponding to neither that of the open chain thiosemicarbazones nor that of the *as*-triazines, indicating the possibility that the only chromophore present is a C=N linkage. The spectral characteristics of the *as*-triazines and thiosemicarbazone derived from valine are shown in Table II; those of the other *as*-triazines corresponded quite closely.

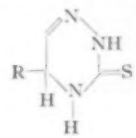
Infrared absorption data were obtained from the thiono-*as*-triazine resulting from leucine. Characteristic bands were shown for C=S at 1,490 cm.$^{-1}$, N—H (associated) at 3,470, 3,390, and 3,100 cm.$^{-1}$, and C—H at 2,960 cm.$^{-1}$ There was no evidence from the small sample used of the enolic mercapto (—SH) group. The spectral bands, however, were sharp and well defined, indicating a pure compound.

BIOLOGICAL ACTIVITY

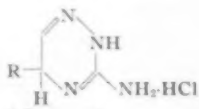
Very few *as*-triazines have been screened for biological activity. Raiziss (8), however, tested a series of N-heterocyclic sulfanilamides including an *as*-triazine derivative which showed low activity against pneumococcal infections in comparison to sulfapyrazine. Fusco (9) reported the preparation of a series of mercapto-*as*-triazines which, in general, were less toxic but also less active against tubercle bacilli than *p*-acetamidobenzal thiosemicarbazone. Dittman (10) found no inhibitory action against Ehrlich carcinoma with 3,5-dioxo-6,6-dimethyl-1,2,-4-triazine, and Kuhn (11) found that several phenolic *as*-triazines showed about one-half the antibacterial activity of 2,2′-dihydroxybenzil. Fusco (12), however, did find that dioxo-*as*-triazines with similar substituents to the active barbiturates possessed appreciable anticonvulsant activity. On the other hand, dihydro-*s*-triazines have shown marked antimalarial, antivitamin, and antitumor activity (13).

Several of the *as*-triazines described in this paper were tested for antiviral activity and found ineffective in mice infected with influenza and encephalitis type viruses. The suspected polymer obtained from leucine was also found ineffective. The acute intraperitoneal toxicities of these compounds in

TABLE I.—5-ALKYL-2,5-DIHYDRO-*as*-TRIAZINES



R	M. p.[a]	Yield, %[b]	Recrystn. Solvent	Formula	Analyses, % Calcd.	Found[c]
Isobutyl	90–91°	18	Aq. ethanol	$C_7H_{13}N_3S$	C: 49.08	49.08
					H: 7.65	7.63
Isopropyl·H_2O	189–190°	11	Aq. ethanol	$C_6H_{11}N_3S·H_2O$	C: 41.11	40.82
					H: 7.48	7.21
					H_2O: 10.28	9.98
Benzyl·H_2O	208–210°	16	Aq. ethanol	$C_{10}H_{11}N_3S·H_2O$	C: 53.85	53.54
					H: 5.87	5.58
					H_2O: 8.07	7.80



Isobutyl	185–187°	23	1-Propanol	$C_7H_{15}N_4Cl$	C: 44.08	43.86
					H: 7.93	8.11
Isopropyl	143–145°	12	1-Propanol-benzene	$C_6H_{13}N_4Cl$	C: 41.26	40.90
					H: 6.35	6.20
Methyl·$4H_2O$	135–137°	..	1-Propanol-benzene	$C_4H_9N_4Cl·4H_2O$	C: 21.75	21.87
					H: 7.69	7.19
					H_2O: 32.61	31.95

[a] The melting points were taken on a Fisher-Johns block and are uncorrected.
[b] The yields are based on the quantity of α-phthalimidoaldehyde thiosemicarbazone used, and therefore should not exceed approximately 50%.
[c] The analyses for carbon-hydrogen were obtained from Weiler and Strauss Microanalytical Laboratory, Oxford, England.

mice were comparatively high, with regard to those of the aryl-substituted mercapto-*as*-triazines reported by Fusco (9). Whereas he reported oral toxicities in mice of 2,000 mg./Kg., our compounds showed intraperitoneal toxicities ranging from 20–200 mg./Kg. The polymer, however, showed a toxicity of 1,666 mg./Kg.

EXPERIMENTAL

Thiosemicarbazone Formation.—α-Phthalimidoaldehyde (1) (0.02 mole) was dissolved in 50 ml. of aqueous ethanol and treated with an equivalent amount of thiosemicarbazide and 0.5 ml. of acetic acid. The mixture was warmed for two hours on a steam bath, cooled, filtered, and the product was recrystallized from 95% ethanol. Yields of 82 to 97% were obtained of thiosemicarbazones which melted according to the reported values (14).

5-Alkyl-4,5-dihydro-*as*-triazine-3(2H)-thiones.—The procedure of Emerson (15) for hydrazinolysis of phthaloyl groups was utilized. Water (100 ml.), α-phthalimido aldehyde thiosemicarbazone (0.02 mole), and hydrazine (0.04 mole) were magnetically stirred at 100° until solution was complete, at which time a red oil separated. The supernatant solution, after removal of the oil, was acidified to pH 3 with dilute hydrochloric acid and extracted with ether. Evaporation of the ether extract gave yellow solids which were recrystallized from aqueous ethanol. Attempts to recrystallize the oils gave noncrystalline semisolids.

TABLE II.—ULTRAVIOLET ABSORPTION SPECTRA OF THE 5-ISOPROPYL-2,5-DIHYDRO-*as*-TRIAZINES AND RELATED COMPOUNDS

Compound	λmax. (mμ)[a]	ε max
5-Isopropyl-4,5-dihydro-*as*-triazine-3(2H)-thione	348	11,460
	247	10,920
5-Isopropyl-3-amino-2,5-dihydro-*as*-triazine hydrochloride	268	7,570
	235	5,470
5-Isopropyl-3-amino-*as*-triazine	241	22,790
2-Phthalimido-4-methylbutanal thiosemicarbazone	274	28,300
	222	57,990
Phthalimide	270	19,820
	238	15,450
	229	18,550
Polymer	231	...

[a] The spectra were obtained with a Cary Automatic Recording Spectrophotometer using ethanol as solvent.

3-Amino-5-alkyl-2,5-dihydro-*as*-triazine Hydrochlorides.—The aqueous residue from the previous procedure, after ether extraction, was filtered to remove the precipitated phthalhydrazide. It was then concentrated *in vacuo*, and the residue was dried in a desiccator and recrystallized from a suitable solvent. In the case of 3-amino-5-methyl-2,5-dihydro-*as*-triazine hydrochloride tetrahydrate, the extremely hygroscopic material was separated from the recrystallization solvent by decantation and immediately covered with anhydrous ether. The ether was evaporated from the product in a vacuum desiccator.

3-Amino-5-isopropyl-*as*-triazine.—To 0.17 Gm. (0.001 mole) of 3-amino-5-isopropyl-2,5-dihydro-*as*-triazine in 5 ml. of water was added sufficient 10% sodium carbonate solution to raise the pH to 8. The white precipitate was collected and recrystallized from 95% ethanol, giving 0.1 Gm. (71% yield) of 3-amino-5-isopropyl-*as*-triazine. The colorless needles melted at 177–178°.

Anal.—Calcd. for $C_6H_{10}N_4$: C, 52.15; H, 7.30. Found: C, 51.98; H, 7.46.

SUMMARY

1. Hydrazinolysis of α-phthalimido aldehyde thiosemicarbazones resulted in the formation of both 3-thiono- and 3-amino-5-alkyl-2,5-dihydro-*as*-triazines, as well as polymeric material. Monoalkyl-substituted *as*-triazines have not been previously reported.

2. Several of the *as*-triazines were tested for antiviral activity in mice and found to be ineffective. The compounds tested were: 5-isobutyl-3-amino-2,5-dihydro-*as*-triazine hydrochloride, 5-isopropyl-3-amino-*as*-triazine, 5-isopropyl-4,5-dihydro-*as*-triazine-3(2H)-thione monohydrate, and the polymer of 2-amino-4-methylpentanal thiosemicarbazone.

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Plasma Levels of Mephenesin, Mephenesin Carbamate, Guaiacol Glyceryl Ether, and Methocarbamol (AHR-85) After Oral and Intravenous Administration in the Dog*

By ANN M. MORGAN,† EDWARD B. TRUITT, Jr.,† and J. MAXWELL LITTLE

A modification of the chromotropic acid method for mephenesin has been used to determine the plasma concentrations of mephenesin, mephenesin carbamate, guaiacol glyceryl ether, and a new compound, methocarbamol (AHR-85), 3-(*o*-methoxyphenoxy)-1, 2-propanediol monocarbamate. The rate of absorption of all of these compounds in the dog has been measured by plasma concentration determinations following oral administration. Also, the rate of disappearance from the plasma of guaiacol glyceryl ether and AHR-85 has been measured following intravenous administration. The oral absorption curves demonstrate a prolongation of elevated plasma concentrations for the two carbamates. The absence of free guaiacol glyceryl ether in the plasma after oral AHR-85 administration indicates that the intact molecule of this carbamate is the active agent in the dog.

Since the introducion of mephenesin, 3-(*o*-methylphenoxy)-1, 2-propanediol, by Berger and Bradley (1), there has been a continuing interest in various substituted ethers of glycerin which might have a longer duration of action as central nervous system depressants without the hemolytic effect. Ginzel (2) and Ginzel, *et al.* (3) compared the pharmacologic activity of a closely related analog, guaiacol glyceryl ether, 3-(*o*-methoxyphenoxy)-1,2-propanediol (GGE), and found similar depressant action but a much weaker hemolytic effect. This has been confirmed by Berger, *et al.* (4). Other studies by Berger (5) and Richardson, *et al.* (6) have reported that the monocarbamate ester of mephenesin[1] has a somewhat longer action, but the insolubility of the compound has precluded any hemolytic studies. Truitt and Little (7) have found that the monocarbamate of guaiacol glyceryl ether (methocarbamol, AHR-85), 3-(*o*-methoxyphenoxy)-1,2-propanediol monocarbamate,[2] has a prolonged pharmacologic action, but much less hemolytic activity (8).

Several analytical methods have been modified to determine the absorbability of these substituted glyceryl ethers in plasma (Wyngaarden, *et al.* (9), Titus, *et al.* (10), and Dresel, *et al.* (11)). These methods depend upon either coupling of the phenolic ethers with a reactive diazonium compound, or upon periodate oxidation of the number one carbon of the glyceryl chain to formaldehyde and colorimetric determination with chromotropic acid.

EXPERIMENTAL

The use of modifications of the chromotropic acid (CTA) method has made it possible to measure the plasma concentrations of mephenesin, mephenesin carbamate, GGE, and AHR-85 by the same analytical procedure. The CTA method was chosen as the most suitable for all of these compounds for two reasons. First, while the coupling method is a sensitive measure for mephenesin, it is not so for GGE. Even though the CTA method is less sensitive, a close correlation for mephenesin has been obtained between the coupling and CTA methods, except at low plasma levels, in this laboratory (Table I) and as reported by Titus, *et al.* (10).

TABLE I.—ANALYSIS OF PLASMA CONCENTRATIONS OF MEPHENESIN IN TWO DOGS BY BOTH COUPLING AND CHROMOTROPIC ACID METHODS AFTER 100 MG./KG. I. V.

	Mcg./ml.	
	CTA	Coupling
Dog A		
1	48.0	49.8
2	26.5	28.0
3	9.9	15.5
4	4.1	6.5
Dog B		
1	57.5	55.0
2	47.0	50.0
3	33.7	28.0
4	17.8	18.0
5	3.0	4.0

* Received January 24, 1957, from the Laboratory of the A. H. Robins Company, Inc., and the Department of Physiology and Pharmacology, The Bowman Gray School of Medicine of Wake Forest College, Winston-Salem, N. C.

† Present address: Department of Pharmacology, University of Maryland School of Medicine, Baltimore 1.

[1] Generously supplied as Tolseram by Dr. C. R. Linegar, E. R. Squibb & Sons, New Brunswick, N. J.

[2] Synthesized by Dr. Robert S. Murphey, A. H. Robins Company, Inc., Richmond, Va.

Secondly, the CTA method measures the free drug before alteration of the 1 carbon, which Riley and Berger (12) described as the initial point of metabolic alteration for mephenesin. This route of metabolism probably is true for the other compounds as well.

Method for Mephenesin and GGE.—For these two compounds the method described by Titus, *et al.* (10) was used almost without modification[3] for the actual extraction and determination from plasma. However, the results were read from a standard reference curve as μg./ml. Samples containing known amounts of the drugs in plasma[4] ranging from 10–100 μg./ml. were determined by the above procedure, and the results plotted as μg./ml. of drug *vs.* per cent transmission on semilog paper. Linear graphs are possible through these concentrations.

Method for Mephenesin Carbamate and AHR-85.—The method for the determination of the carbamates of mephenesin and GGE is a modification of the above procedure. It utilizes an alkaline hydrolysis for removal of the carbamate ester group before the periodate oxidation to formaldehyde and colorimetric determination with chromotropic acid.

Procedure.—Place 1.0 ml. of plasma plus 0.1 ml. of 1*N* NaOH in a 15-ml. graduated centrifuge tube, stopper loosely with a ground-glass stopper and place in a boiling water bath for exactly ten minutes hydrolysis. If the volume has diminished, replete with distilled water. The tubes are removed, cooled to room temperature, and the contents are washed into glass-stoppered centrifuge bottles with 25 ml. of redistilled chloroform. The remaining procedure is that of Titus, *et al.* (10). Samples covering the same range of concentrations as for mephenesin and GGE are carried through the modified procedure. The results are plotted as μg./ml. *vs.* per cent transmission on semilog paper. Linear graphs are possible for these compounds, also, through this range of concentrations.

The recovery of mephenesin from mephenesin carbamate and GGE from AHR-85 is not 100% of the theoretic yield, but is 60% for mephenesin carbamate and 80% for AHR-85 over this range of plasma concentrations (Tables II and III). However, the experimental recovery values are read from an extracted standard curve, so a factor is not necessary to raise these values to 100%.

[3] Two normal $NaAsO_2$ was used instead of the 1 *N* recommended by Titus, *et al.* (10).

[4] Plasma should be free from added dextrose, as large amounts of this substance cause significant interference due to charring. This was determined in this laboratory and also reported by MacFadyen (13).

TABLE II.—AVERAGE RECOVERY OF MEPHENESIN CARBAMATE FROM PLASMA

Mcg./ml.		
Added	Recovered	Recovery, %
20.0	13.1	65.5
25.0	15.4	
	13.7	58.1
50.0	30.6	62.6
	32.1	
60.0	35.2	58.8
75.0	46.5	60.8
100.0	57.4	57.4
	57.4	
		Av. = 60.5 ± 3.1– S. D.

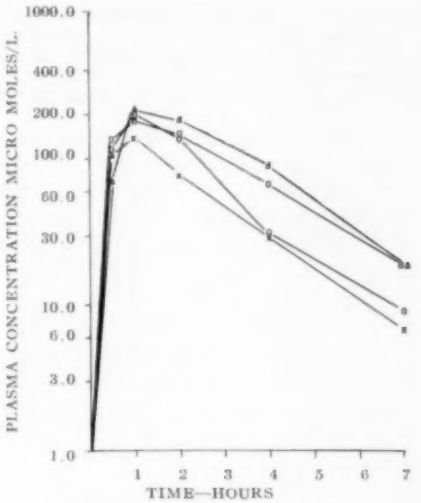

Fig. 1.—Average plasma level after oral doses equivalent to 100 mg./Kg. of mephenesin.

×—× Mephenesin (10 dogs)
□—□ Mephenesin carbamate
○—○ Glyceryl guaiacolate } (8 dogs)
△—△ AHR-85

RESULTS

The mean absorption curves for mephenesin, mephenesin carbamate, GGE and AHR-85, following oral administration of doses equimolar to 100 mg./Kg. of mephenesin, are shown in Fig. 1. These are

TABLE III.—AVERAGE RECOVERY OF AHR-85 FROM PLASMA

Mcg./ml.		
Added	Recovered	Recovery, %
12.5	8.9	
	9.7	74.2
	9.2	
25.0	19.8	
	20.0	80.8
	20.7	
50.0	37.8	
	38.3	77.4
	38.8	
	39.8	
75.0	56.5	
	66.2	84.1
	66.6	
100.0	80.2	
	83.2	
	74.0	79.8
	79.0	
	82.5	
		Av. = 79.3 ± 4.7– S. D.

based upon 10 dogs for mephenesin and 8 dogs for the other drugs. The doses were administered in gelatin capsules to unfed animals, and blood levels were measured at zero, one-half, one, two, four, and seven hours by the CTA method. Food, but not water, was withheld during the experiment. There appears to be a significant elevation of GGE at the second hour, of mephenesin carbamate at the second and fourth hour, and of AHR-85 at the first and second hour, when the mean concentration is compared to the mean mephenesin concentration using "Students" *t* test. However, with the exception of the first half hour, the highest plasma concentrations were obtained with AHR-85. The seventh hour plasma levels were not included in the statistical analysis because of the extreme range of the determinations (Table IV).

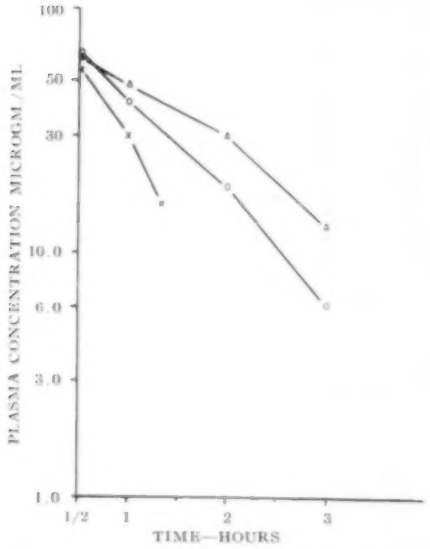

Fig. 2.—Average plasma levels after i. v. doses.

×—× Mephenesin 100 mg./Kg. (Titus, *et. al.* Data—5 dogs)
△—△ AHR-85 121.5 mg./Kg. (6 dogs)
○—○ Guaiacol, glyceryl ether, 100 mg./Kg. (6 dogs)

Figure 2 shows blood levels *vs.* time following the intravenous administration of doses of AHR-85 and GGE equimolar to 100 mg./Kg. of GGE. These levels were determined as μg./ml and compared with several points which show the rate of mephenesin disappearance from plasma following an intravenous dose of 100 mg./Kg., as determined by Titus *et al.* (10).

This method has also been applied to plasma samples containing AHR-85 before the hydrolysis procedure. In plasma obtained from six dogs during the first two hours after the administration of AHR-85, some GGE was demonstrated (2–4% of the hydrolyzed plasma level). Perhaps this is due to slight hydrolysis of the carbamate ester linkage by 1 *N* NaOH during the first extraction step. To test this possibility AHR-85 and mephenesin carbamate were added to pooled plasma. The recovery of the free compounds was 1–3% of the hydrolyzed recovery.

DISCUSSION

The low percentages of free plasma GGE found in six dogs seem to indicate that AHR-85 is largely active as an intact molecule. This is in agreement

TABLE IV.—MEAN PLASMA LEVELS OF MEPHENESIN, MEPHENESIN CARBAMATE, GGE, AND AHR-85 AFTER ORAL ADMINISTRATION TO DOGS. (ALL DOSES EQUIVALENT TO 100 MG./KG. MEPHENESIN)

	Plasma Concentrations—microMoles/L. Time, hours					
Drug	½	1	2	4	7	No. of Dogs
Mephenesin	107.1	140.2	78.86	30.73	6.64	10
S. D.	90.98	67.70	32.36	23.0	(1.1–22.0)[a]	
GGE	118.17	205.84	141.91	33.08	9.14	8
S. D.	94.49	93.13	53.53	16.87	(5.6–27)[b]	
t value	0.24	1.64	2.94	0.23		
p value	0.84	0.12	0.01[e]	0.82		
Mephenesin Carbamate	132.50	186.8	148.75	68.25	18.62	8
S. D.	122.0	103.1	72.20	25.70	(10–30.3)[c]	
t value	0.48	1.096	2.16	3.11		
p value	0.65	0.28	0.018[e]	0.01–0.001[e]		
AHR-85	70.73	216.55	184.41	90.63	18.57	8
S. D.	29.89	52.16	84.50	90.51	(6.0–108.0)[d]	
t value	1.025	2.49	3.46	1.924		
p value	0.35	0.02[e]	0.01–0.001[e]	0.07		

[a] Range—6 out of 10 dogs had meas. levels.
[b] Range—5 out of 8 dogs had meas. levels.
[c] Range—7 out of 8 dogs had meas. levels.
[d] Range—3 out of 8 dogs had meas. levels.
[e] Significant—$p \leq 0.05$.

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{\Sigma(x_1 - \bar{x}_1)^2 + \Sigma(x_2 - \bar{x}_2)^2}{N_1 + N_2 - 2}} \times \frac{N_1 + N_2}{N_1 N_2}}$$

with Berger (5), Richardson, *et al.* (6), and Dresel, *et al.* (14) who claimed a similar activity for the unhydrolyzed molecule of mephenesin carbamate. These investigators based their conclusions on the greater activity of mephenesin carbamate and its urinary excretion as the intact ester. Recent studies in humans have confirmed the presence of the intact ester in plasma (15).

The significantly extended blood levels of the two carbamates are apparently not the result of slow intestinal absorption in the dog since the one-half hour concentrations were not significantly different. Pharmacologic evidence reported elsewhere (7) also substantiates the extended activity of AHR-85 compared to mephenesin and GGE.

When the plasma concentrations are compared with those for mephenesin following equimolar doses, the second hour concentration of GGE is significantly elevated, as are the second and fourth hour concentrations for mephenesin carbamate and the first and second hour concentrations of AHR-85. The highest values were obtained with AHR-85. The slight recovery of free GGE after the oral administration of AHR-85 suggests that it is the intact molecule of AHR-85 which is active in the dog.

SUMMARY

The chromotropic acid method for the determination of mephenesin has been modified to determine guaiacol glyceryl ether (GGE) and the carbamate esters of these two compounds: mephenesin carbamate (Tolseram) and methocarbamol,3 - (*o* - methoxyphenoxy) - 1,2 - propanediol—monocarbamate (AHR-85). The method applied to the two carbamate esters gives partial, but quantitatively reproducible, recoveries. The rate of absorption has been measured by the determination of plasma levels for all four compounds following oral administration, and for GGE and AHR-85 following intravenous administration.

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Haze Formation of Rubber Closures for Injections*

By GEORGE MILOSOVICH† and ALBERT M. MATTOCKS

An investigation of the use of nephelometry in evaluating rubber closures and washing procedures is reported. Evidence is presented that a nephelometric measure of haze can be of value in comparing closures, solutions, and washing procedures for production of haze. The closures tested varied in their property of producing haze and sodium bisulfite and acetic acid solutions were found to prevent haze formation. An acetone wash was shown to reduce haze considerably, indicating that haze is partly due to sulfur present in the closures.

PROBABLY THE most common reason for rejection of multiple-dose parenteral solutions after packaging is the appearance of a fine colloidal precipitate or haze in the solution. Although the haze is sufficient to be objectionable, the amount of material it constitutes is extremely small. Such materials may be the result of reaction between injection solutions and ingredients of the rubber, or, more likely, are insoluble components of the rubber such as fillers, mold release agents, or sulfur which have been mechanically removed.

If a method were available which would give a reliable measure of haze formation it would be of

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considerable value as an aid in predicting incompatibilities of this type between closures and injection solutions. Also, it might be used to evaluate washing procedures for closures as to their effectiveness in removing insoluble substances from closure surfaces.

Methods based on nephelometry have been used to evaluate new closure formulations for their haze production when autoclaved in the presence of water (1, 2, 3). Morrisey and Hartop (4) have recently published a test procedure involving the use of nephelometry and claim that the number of rejected lots of parenteral preparations due to off-quality closures has been sharply reduced since its adoption as a control procedure.

These methods are believed to be inappropriate for predicting stability of a new combination of closure and solution, since the test procedure itself is believed to induce chemical changes in the rubber. When treated at high temperatures over a period of time the closures are likely to be oxidized or softened and thus exhibit properties not normally present. These methods also use only distilled water as a test medium, and one cannot evaluate the effects to be expected when a solution is in contact with the rubber.

The procedure of treating closures reported in this paper is believed to be much less drastic than those previously used. This procedure also allows the use of solutions as well as water since the closure-medium combinations are sealed. Using this procedure the effect of several chemicals on haze formation and the evaluation of an acetone wash were determined.

EXPERIMENTAL

The closures tested in this investigation were West Company Gum 79, Pink 124, Red 87, Red 123, White 86, Black 210, and Black 260. The solutions were 0.5 *M* or 1% and made with triple-distilled water and reagent grade chemicals. It was thought that the rate of haze formation could be used to compare various closures and treatments and thus a measure of haze formation with time was needed.

Pyrex test tubes of suitable size (18 x 150 mm.) were washed on an ampul washer with six cycles of steam and distilled water and dried at 100°. The closures were agitated in hot distilled water for 15 minutes, rinsed with hot distilled water, autoclaved in the presence of distilled water for 30 minutes, rinsed again in hot distilled water and dried over silica gel.

One closure was placed in each tube along with 10 ml. of the solution to be tested and the tubes were sealed by flame. They were then incubated in an oven at 60° and their turbidities measured at the end of one-half, one and one-half, three, six, seventeen, and thirty days on a Coleman Model 9 nephelometer. Sixty degrees was chosen, since the rate of haze formation at this temperature was high enough to give readings in short periods of time while the closures exhibited no visible evidence of aging.

After completing initial experiments it was found that the variation between individual closures of the same type was quite large. It was felt that using a group of closures rather than single ones would reduce this variation and a special cell which would hold 20 or more closures was constructed to facilitate this. It is shown in Fig. 1.

RESULTS

Nephelometric readings generally increased with time to a maximum and then leveled off or dropped. These changes were frequently accompanied by formation of visible precipitates. It is believed that even before these precipitates were formed many of the particles in suspension agglomerated to give inconsistent readings. The earlier values seemed to be free of these changes and these were considered to be useful as a means of comparison. For this purpose readings taken after six days were selected. The results, expressed as nephelos units, are shown in Table I and the analysis of variance of the results is shown in Table II.

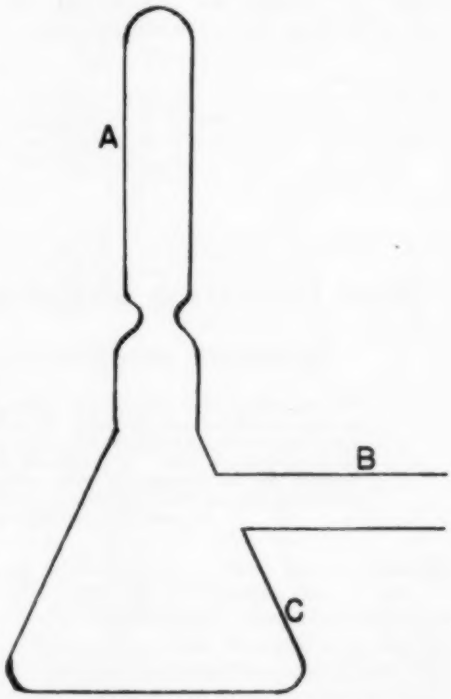

Fig. 1.—Special flask for nephelometric measurements. A, 18 x 150-mm. Pyrex test tube; B, 19-mm. Pyrex tube; C, 125-ml. Erlenmeyer flask.

TABLE I.—RESULTS OF NEPHELOMETRIC MEASUREMENTS: SINGLE CLOSURE TUBES

Solution	Turbidity-Nephelos Numbers, Duplicate Determinations — Rubber Composition — Gum 79	Pink 124	Red 87	White 86	Black 210	Red 123	Black 260
Water	2.7	36.6	5.0	11.0	65.8	25.5	12.5
	5.0	39.7	8.0	10.6	41.5	5.0	4.9
0.5 *M* Na_2SO_4	4.9	13.2	3.4	1.9	58.3	1.3	3.4
	3.7	4.7	0.9	3.4	75.4	3.4	3.0
0.5 *M* $MgSO_4$	1.9	10.2	2.4	2.8	86.1	3.2	4.2
	0.9	12.4	2.5	1.2	95.0	3.9	3.9
0.5 *M* $NaHSO_3$	2.0	2.1	0.3	1.5	0.1	1.6	0.3
	6.7	−5.8	−1.0	−1.1	−2.1	−1.8	−5.6
1% acetic acid	1.6	4.9	10.1	1.7	−0.2	0.0	0.4
	4.0	9.6	2.1	2.9	0.7	3.3	2.1

From the analysis of variance it is seen that there is a difference in haze formation shown by the different closures, and this difference is due primarily to the higher haze formation caused by Black 210 and Pink 124. A significant composition × solution interaction was found and shown to be due almost entirely to the fact that Black 210 gave considerably higher haze than the other closures with water, sodium sulfate, and magnesium sulfate solutions but no higher with bisulfite or acetic acid solutions.

Haze formation was about equal in water and magnesium sulfate solution, slightly lower in sodium sulfate solution, and practically zero in sodium bisulfite and acetic acid solutions.

Proceeding further on the hypothesis that haze is due to surface particles on the closure, it was believed that these insoluble particles were mainly sulfur or insoluble zinc salts. Preliminary experiments followed by microscopic examination of closure surfaces indicated that sulfur particles on the surface might be removed rather easily by acetone. Consequently, closures were placed in flasks of reagent acetone and kept at 60° for five hours, rinsed three times with fresh portions of acetone and dried in a vacuum desiccator. Prior to the acetone wash these closures had been washed as described above, as had the "untreated" closures of this experiment. Twenty closures along with 100 ml. of water were placed in one of the special flasks which was then sealed. One flask containing each type of treated closure was run along with one containing each type of untreated closure. They were incubated at 60° and read in the nephelometer at intervals of time.

The haze developed at different rates with the various closures, so that no particular set of values seemed suitable for comparison. Results are best shown by graphs such as those of Figs. 2, 3, and 4. Examination of the graphs shows clearly that the haze formation was reduced considerably as a result of the acetone treatment.

DISCUSSION

Nephelometric type measurements are subject to several variables which are difficult to control. The particle size of the suspended material must be uniform and of the same size range in the various samples to be compared. The thickness of the nephelos cells as well as the diameter must be uniform, although this is less critical.

No attempt was made in this investigation to

TABLE II.—RESULTS OF NEPHELOS MEASUREMENTS: SINGLE CLOSURE TUBES

Source	Analysis of Variance s.s.	d.f.	m.s.	F	F/I
Composition (C)	12,439.98	6	2,073.33	72.6[a]	5.94[a]
$6C_{210}$-C_{124}-C_{123}-C_{86}-C_{87}-C_{79}-C_{260}	11,718.43	1	....	410.5[a]	33.5[a]
$5C_{124}$-C_{123}-C_{86}-C_{87}-C_{79}-C_{260}	706.87	1	....	24.8[a]	
Solution (S)	4,100.97	4	1,025.24	35.9[a]	2.94[a]
$4S_4$-S_3-S_2-S_1	1,954.12	1	....	68.4[a]	
$3S_4$-S_3-S_2-S_1	1,838.11	1	....	64.4[a]	
$2S_3$-S_2-S_1	242.08	1	....	8.48[a]	
S_2-S_1	66.66	1	....	2.33	
Replicate	22.51	1		<1	
C × S	11,768.38	24	349.17	12.2[a]	
S×C_{210}-C_{124}-C_{123}-C_{86}-C_{87}-C_{79}-C_{260}	10,604.39	4	2,651.10	92.9[a]	
$4S_5$-S_4-S_3-S_2-S_1	3,477.31	1		121.8[a]	
$3S_4$-S_3-S_2-S_1	5,261.71	1		184.2[a]	
$2S_3$-S_2-S_1	1,406.34	1		49.3[a]	
S_2-S_1	459.03	1		16.1[a]	
C × R	42.02	6	7.00	<1	
S × R	156.23	4	39.06	1.37	
Residual	685.18	24	28.55		
TOTAL		69			

NOTE: S_1—water, S_2—$MgSO_4$, S_3—Na_2SO_4, S_4—acetic acid, S_5—$NaHSO_3$.
[a] Significant at 5 per cent or lower level.

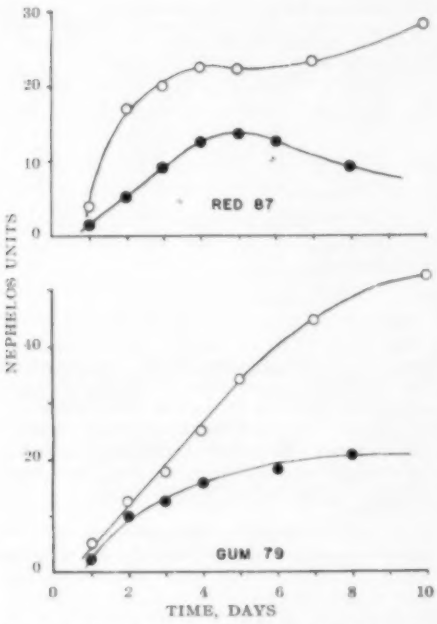

Fig. 2.—Effect of acetone wash on haze formation, —○—, untreated; —●—, acetone washed.

control the particle size of the material. It was felt that there would be a direct relationship between nephelometric readings and what would be visible to the eye. However, this relationship will not hold when the particles are of large enough dimensions to settle. Attempts are now being made to prevent the formation of large particles and the results of this investigation will be presented at a later date. An initial experiment showed that the error due to variation in the test tubes was ±0.8 nephelos units, which is small enough to be neglected.

The measurement of haze formation by the procedure described showed that none of the closures formed an appreciable amount of haze when immersed in sodium bisulfite or in acetic acid solution. Haze is believed to be mainly the formation of colloidal solutions of zinc salts or sulfur. Zinc oxide is a common closure ingredient, and zinc stearate is widely used as a lubricant during closure manufacture. Other zinc salts may be present as the result of reactions taking place during vulcanization. Sulfur is an ingredient of all compositions, and it is commonly known to diffuse to the surface, a process termed blooming. The other insoluble materials such as barium sulfate and clay might also be expected to form colloidal solutions if removed from the closure. The acetic acid may have prevented haze formation by either precipitating the colloid, due to hydrogen ion, or by dissolving zinc salts to form soluble zinc acetate. Sodium bisulfite may have acted in the same manner, precipitating the colloid or by reacting with sulfur. The fact that both compounds prevented haze formation should have immediate practical application and is certainly deserving of further study.

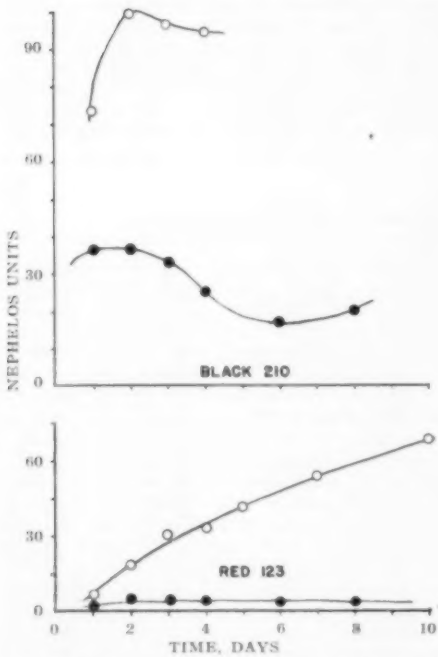

Fig. 3.—Effect of acetone wash on haze formation, —○—, untreated; —●—, acetone washed.

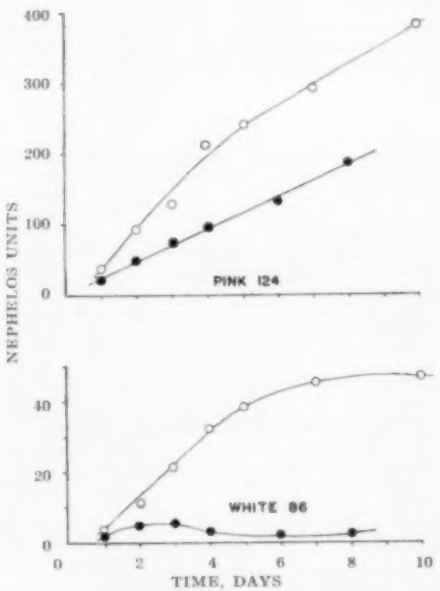

Fig. 4.—Effect of acetone wash on haze formation, —○—, untreated; —●—, acetone washed.

The procedure of washing closures in acetone before use, shown to reduce haze formation, can be applied directly in manufacturing plants with little extra effort. In fact, such a technique is less tedious than most pretreatments now used. The fact that sulfur is soluble in acetone, and acetone washing reduced haze, would indicate that much of the haze is due to sulfur which is removed from the closure by the solution in contact with it.

Much work is necessary before the true value of the nephelometric test for haze can be ascertained. However, the findings reported in this paper give every indication that such a test will be of definite value in selecting solutions, rubber closures, and washing procedures in such combinations so as to give products in which haze formation is at a minimum.

SUMMARY

1. Evidence is presented that a nephelometric test can be of value in comparing closures, solutions, and washing procedures for production of haze.

2. The closures tested varied in their property of producing haze, with Black 210 and Pink 124 giving the greatest amount, and Gum 79, Red 87, and White 86 the least.

3. Sodium bisulfite and acetic acid solutions were found to prevent haze formation.

4. An acetone wash was shown to reduce haze considerably, indicating that haze is partly due to sulfur present in the closure.

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Carvone Analysis by Ultraviolet Absorption*

By ROBERT H. REITSEMA† and WILLIAM E. FAAS

An ultraviolet absorption analysis of carvone in spearmint oil or in other solvents is presented. The method was designed as a rapid assay using the minimum amount of expensive spectrophotometric equipment without sacrifice of significant accuracy.

THE ANALYSIS of carvone in spearmint oil is traditionally performed by the bisulfite method. The assay is based upon the solubility of carvone in sodium bisulfite solution (1). The insoluble noncarvone portion is measured visually and carvone is calculated by difference. It is not a particularly convenient assay method despite the advantages of mechanical shakers and continuous heating devices. Thus, a more accurate and a simpler method was desired.

It is recognized that the bisulfite method is not completely specific for carvone. The readings themselves are accurate only to about one per cent carvone. Materials other than carvone can either react with the bisulfite as do other aldehydes and ketones, or dissolve or be suspended in the solution as does at least one per cent of noncarvone spearmint oil. These then would be assayed as carvone. The reversibility of the reaction of bisulfite with carvone means that some of the carvone is out of solution at all times and thus is not reported as carvone. These errors can be most significant in the assay.

Polarography, ultraviolet absorption, and infrared absorption were three possible instrumental methods of analysis for carvone, and the properties of carvone with each of these is well established. The easiest of the three to apply to routine assays in the greatest number of laboratories appeared to be ultraviolet absorption. Polarography, because of problems associated with its use in rapid routine analyses, was not so attractive. Infrared absorption analysis of carvone in spearmint has been found to be useful. It was possible to obtain good results using whole spearmint oil without dilution at the 1100 cm.$^{-1}$ peak. Work at the major point at about 1675 cm.$^{-1}$ would require preparation of solutions. The assay of carvone by the infrared method

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appeared completely feasible and could be used. The equipment is not always available, however, and in view of the success with the near ultraviolet region, infrared seemed to offer no real advantage at the present time.

Carvone shows a main absorption band at 235 mμ and this has been proposed as a point for the ultraviolet analysis of carvone (2), although no specific data on the use of this band were presented in the preliminary paper. It was found in the current work that the great dilution (1:62,500) required because of the high extinction coefficient of carvone was a hindrance to rapid assays since at least two dilutions of a sample were indicated. A large volume of solvent was needed. There seemed to be no real difficulty with running the assay at this point, but for rapid assays of carvone the absorption at 320 mμ was preferred. This absorption peak has three advantages: a single dilution to a total volume of only 15 ml. was needed to prepare the solutions; a tungsten light source could be used with the spectrophotometer; and the cheaper Corex cells were usable instead of silica ones. Although these advantages were not of themselves too significant, they did make it possible for more laboratories to run the analyses cheaply and rapidly. There seemed to be very little difference in accuracy of the analyses whether performed at 235 or 320 mμ on the basis of several samples of spearmint run at both points.

EXPERIMENTAL

A Model DU Beckman spectrophotometer was used in these studies with 1-cm. path Corex cells. The selector switch was set at 0.1 thereby permitting use of more concentrated solutions for the analysis. For the routine assay study, matched Corex cells with the tungsten light source were used. Comparison with silica cells and hydrogen lamp showed little difference in accuracy and therefore the procedure with the less expensive accessories was accepted.

A sample of 0.1 ml. of spearmint oil (or carvone containing solution) was measured with a micro blood sugar pipet. This sample was diluted with 15.0 ml. of A. C. S. grade methanol added by a pipet. The solution was then used to fill a 1-cm. Corex cell and the absorbance at 320 mμ was read. From the standard chart was then obtained the corresponding per cent carvone.

DISCUSSION

A standard curve was prepared from solutions of pure *l*-carvone in methanol, in limonene, and in a head fraction of a spearmint distillation. These three series were used to study the accuracy of the method and to evaluate some of the interferences which might be encountered. It was found that the results of the three series could be plotted on the same graph and a straight line drawn through the points. The per cent carvone found by the analysis was in all cases within 1% of the theoretical value. This, then, was the total experimental error under conditions for routine analysis. This error includes the measurement of 0.1 ml. of oil in a micro pipet, dilution to 15.0 ml., reading the absorbance, and determination of the carvone percentage from the calibration curve. The results are shown in Table I and Fig. 1.

TABLE I.—ABSORBANCE OF CARVONE IN VARIOUS SOLVENTS

Solvent	Carvone, % v/v	Absorbance	Carvone Found, %
Methanol	60.0	1.059	60.2
	70.0	1.226	70.8
	80.0	1.348	79.2
	90.0	1.504	89.6
Limonene	60.0	1.045	59.3
	65.0	1.128	64.8
	70.0	1.217	70.6
	75.0	1.282	74.8
	80.0	1.372	80.7
Spearmint fraction	64.6	1.132	65.2
	64.6	1.130	65.0
	73.0	1.259	73.1

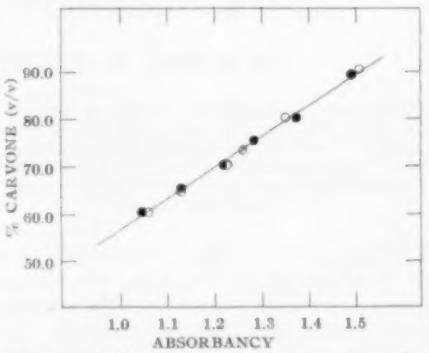

Fig. 1.—Calibration curve for carvone in various solvents: ○, in methanol; ●, in limonene; ⊕, in spearmint terpenes.

The data of Table I were based upon solutions prepared on a volume basis. It was possible to convert the data to a weight basis without significant change in accuracy of the method. Since the industry is accustomed to the volume basis it is this curve which is given, although to many workers the w/w calibration would be more meaningful.

In addition to an absolute carvone assay it was necessary to correlate the ultraviolet absorption assay method with the bisulfite method if possible

This would be of interest to those who still assay spearmint oil by the bisulfite method. It also would give a way of evaluating data accumulated in the past by bisulfite with the information obtained by the new analysis. A purely empirical approach was used. Two hundred and five different samples of spearmint oil were run by both the neutral bisulfite and by the ultraviolet absorption method. The carvone value as determined by the bisulfite method was then plotted against the absorbance of the sample taken under uniform conditions at 320 mμ. As shown in Fig. 2, the points fall on a straight line reasonably well. Because of the differences in the base oils in their behavior both to bisulfite solutions and to absorption in the ultraviolet, the agreement of the two methods is not within the limits of the absolute carvone assay given in Table I. This is shown by the summary of deviations in Table II where the variation of the bisulfite method from the ultraviolet, using the bisulfite based data of Fig. 2, is given for 205 spearmint oils.

TABLE II.—DEVIATIONS BETWEEN BISULFITE AND ULTRAVIOLET SPEARMINT OIL ASSAYS

Deviation Range, %	Number of Samples	Cumulative, %
0–0.5	66	32.2
0.6–1.0	54	58.5
1.1–1.5	42	79.0
1.6–2.0	16	86.8
2.1–2.5	13	93.2
2.6–3.0	7	96.6
Over 3.0	7	100.0

The positive and negative deviations between the bisulfite assay and the ultraviolet assay, using the bisulfite assay as standard, are statistically distributed. Consequently, it has been possible to use the carvone assay obtained from reading absorbance on Fig. 2 and predict satisfactorily the bisulfite assay for a bulking made up from twelve or more individual lots. With this number of samples involved, the two methods agree within 1% by averaging out individual differences. This procedure has been used successfully for the past two years on several hundred samples.

One further comparison between the bisulfite and ultraviolet methods should be noted. Absorbance as has been pointed out can be read on either Fig. 1, where the reference is pure carvone standards, or on Fig. 2, where the reference is the bisulfite assay. The amount of carvone by the data of Fig. 1 would be reported significantly higher. The slopes of the best lines through the points are not quite parallel and the difference between the assays changes gradually from 3.3% higher for the pure carvone standard method at 60% carvone, to 4.9% at 70% carvone. The uniformity of the data of Fig. 1, irrespective of the nature of the solvent, suggested that background absorption of spearmint constituents was not principally responsible for the discrepancy. The incomplete reaction of carvone with bisulfite was more likely. This was checked by running the pure carvone standards in the limonene solvent by the bisulfite method. Xylene was used as a diluent for the preparation of the solutions which were assayed by ultraviolet and

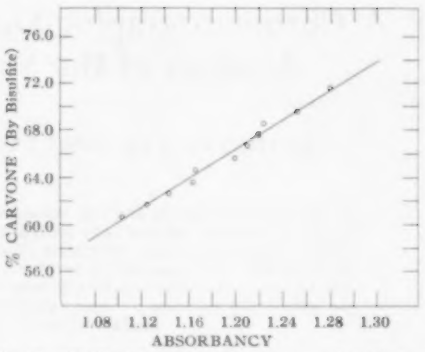

Fig. 2.—Calibration curve for carvone based upon bisulfite assays.

TABLE III.—COMPARISON OF BISULFITE AND ULTRAVIOLET ASSAYS OF KNOWN STANDARDS

Known Carvone, %	Carvone by U. V. (Fig. 1)	Carvone by Bisulfite
65.0	64.8	62.5
70.0	69.7	67.5

bisulfite methods. Results given in Table III show that the bisulfite assay for carvone does not report all the carvone in the sample due to incomplete reaction.

SUMMARY

1. A method for the analysis of carvone in spearmint oil or other solvent has been described. The method appears to be easier, faster, and more accurate than the traditional bisulfite assay. A calibration curve for carvone based upon standard solutions of carvone in three different solvents is given.

2. Correlation of the ultraviolet method with the bisulfite method has been attempted. A separate calibration curve for the ultraviolet absorption based upon actual carvone assay by bisulfite is given. This makes it possible to run assays by ultraviolet and predict with reasonable accuracy the results of the bisulfite assay, particularly the bisulfite ass[illegible] of a bulking of several individual lots. Deviations between the assays for 205 samples of spearmint are summarized.

3. Recommendations that the method of analysis be changed to the ultraviolet method, based on pure carvone standards and the answers be put on a weight basis, are presented.

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A Chromatographic-Densitometric Procedure for Analysis of the Solanaceous Alkaloids*

By TREVOR J. QUINN,† J. G. JEFFREY, and W. C. MacAULAY

A method of analysis for the solanaceous alkaloids employing separation of the major component alkaloids by means of paper chromatography followed by densitometric evaluation of the separated alkaloids has been developed. The method has been applied to a number of products and the results compared with those of the official assay of the British Pharmacopoeia. Results were satisfactory and are felt to be sufficiently accurate for many purposes. It is felt that the process merits further study and more widespread application.

THE METHODS of analysis which are currently official for the solanaceous alkaloids employ a titrimetric determination of total alkaloid content, calculated as hyoscyamine. During recent years there has been a growing dissatisfaction with these procedures and an increasing preference for methods giving specific results for each of the component alkaloids. The search for such methods has been extensive, and many new techniques and analytical instruments have been used in various approaches to the problem (1–5). The most promising method resulting has involved a paper chromatographic separation of the scopolamine from the atropine-hyoscyamine mixture, followed by analysis of the separated alkaloids. This analysis has been carried out most commonly by elution of the separated alkaloids followed by a determination using the Vitali-Morin colorimetric assay (6–8). Further separation of the alkaloids to achieve separate results for atropine and hyoscyamine has not been deemed necessary, since there is probably some degree of racemization accompanying the extraction, and separate results would be meaningless for most practical purposes.

The use of densitometric procedures has found fairly extensive application in the field of assay of amino acids and sugars (9). It was felt that the use of a form of this method in determination of the solanaceous alkaloids would simplify the process by avoiding the elution of the separated alkaloids, and would result in equally if not more accurate determinations. The method of maximum density was chosen for its simplicity.

EXPERIMENTAL

Apparatus and Materials.—For this extraction, the apparatus specified for the official British Pharmacopoeial assay was employed. In addition, a centrifuge was found necessary in order to break troublesome emulsions which formed during the shaking out of the alkaloids from belladonna preparations.

The chromatography was conducted by ascending technique using two rectangular "Shandon Chromatanks" measuring 7 in. x 9 in. x 22 in. These were fitted into an incubator, so that the runs could be carried out at constant, controlled temperature. Sheets of Whatman No. 1 filter paper measuring 6 in. x 20 in. were cut from larger sheets. Five sheets, each holding four spots, were run in each tank at one time. The solvent employed was a water-saturated mixture of 100 parts of *n*-butanol and 5 parts of glacial acetic acid. The alkaloids were spotted out using a 5-λ Kirk type transfer pipet.

The completed chromatograms were dip-treated with modified Dragendorff's reagent as described by Grant and Kennedy (10). A later modification of this reagent by Thies and Reuther (11) has been specifically recommended for dip-treating chromatograms, but no trials were made with this.

Densitometric determinations were made using a Welch "Densichron" Densitometer with the green filter and a 3-mm. aperture. The tungsten lamp was set for maximum intensity and focused to give maximum even illumination. The entire area of the spot was scanned, and the maximum density observed was recorded.

The products assayed in the comparative study are described in Table I.

Procedure

Preparation of Standard Curve.—Standard solutions of each of the three alkaloidal salts, atropine sulfate, hyoscyamine sulfate, and scopolamine hydrobromide, were prepared containing 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 γ/λ in distilled water. Densitometric determinations were made on 10 runs of

* Received from the College of Pharmacy, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.
Abstracted from a thesis submitted by Trevor J. Quinn to the College of Graduate Studies of the University of Saskatchewan in partial fulfillment of the requirements for the degree of Master of Science.
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Grateful acknowledgment is made to the National Research Council (Canada) for financial support of this project.

each concentration of each alkaloid, using the same chromatographic and densitometric procedures described later for the analysis. A standard curve prepared from these data was used with some misgivings in early comparative assays, for it was noted that readings for scopolamine hydrobromide were

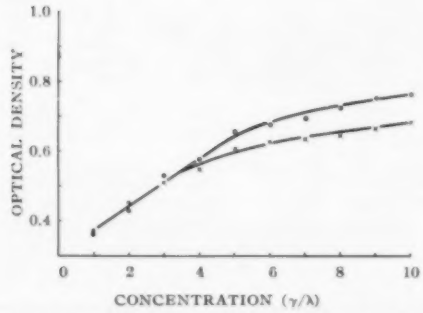

Fig. 1.—Original standard curves (x, atropine-hyoscyamine; o, scopolamine).

almost uniformly higher than those for the other alkaloids. It soon became evident that separate curves for scopolamine and for the atropine-hyoscyamine mixture would be necessary. Further data collected from absorbance readings of marker spots in the comparative assays led to preparation of the curves shown in Fig. 1. The curve for scopolamine was prepared by fitting a line by eye through the plotted means of 50 readings of absorbances representing each of the standard solutions of scopolamine hydrobromide. The other curve employed 40 readings representing each atropine sulfate standard and 10 of each hyoscyamine sulfate standard. The falling off of the curve at higher concentrations was felt to be due to increased trailing of the spots, which was visibly more noticeable with the atropine sulfate and hyoscyamine sulfate, than with the scopolamine hydrobromide. The trailing was not present with the unknown samples, which may have been due to impurities in these samples, or the spotting out of acidic rather than neutral aqueous solutions. Since the trailing did not occur with the unknowns, and since there should be a straight-line relationship between absorbance and concentration, the curve used in the comparative analysis, shown in Fig. 2, was prepared by extrapolating the lower identical portions of the two original curves. Successful reduction of trailing of the standard spots would no doubt yield a more precise curve and give more accurate results.

Comparative Assays.—Two samples of the same product were assayed concurrently by the official procedure outlined in the British Pharmacopoeia. The titrated alkaloids were evaporated to dryness using infrared light and left overnight. The next morning the dry alkaloids were dissolved in an accurately measured quantity of 0.1*N* hydrochloric acid, so as to give a solution containing about 10γ/λ of total alkaloids. The volume of acid required was calculated from the results of the official assay. This solution was used for spotting out on the chromatograms. For each run, 10 spots of 5λ of each of the unknown samples and 10 spots of 5λ of each of two standard solutions were used. As soon as the spots were dry, the sheets were placed in the tanks, to which 300 ml. of the organic layer

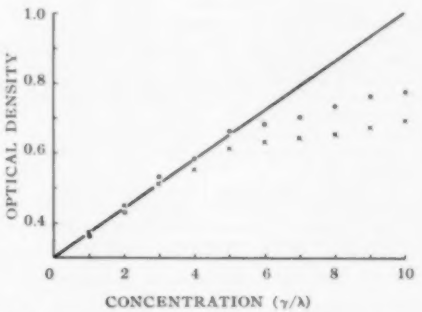

Fig. 2.—Standard curve used in comparative study.

of the *n*-butanol-glacial acetic acid-water mixture had been added previously, and the tanks placed in the incubator at 30°. The chromatograms were run for seven hours and were left to dry overnight. The next morning, the chromatograms were dip-treated with the modified Dragendorff's reagent and again dried. Maximum absorbances of the developed spots were then determined using the densitometer, and the mean value for the ten spots of each unknown was used with reference to the standard curve for evaluation of the concentration of each of the component alkaloids. The content of each alkaloid in the original sample was determined by the following formula:

$$X = VC/10W$$

where

X = per cent alkaloid present in sample.
V = volume (ml.) of 0.1*N* hydrochloric acid used to prepare sample for spotting out.
C = equivalent concentration (γ/λ) from standard curve.
W = weight of sample in grams.

Results for each of the samples tested are presented in Table I.

DISCUSSION

The results presented in Table I indicate a reasonably close agreement between the values obtained by the two methods. Attempts to simplify the method by modifying the extraction and purification procedures, probably along the lines of microextraction, should prove of value. It is felt that the chromatography itself effects some degree of purification of the alkaloids, and that unknowns could be spotted out in a relatively impure form. In a preliminary run to study this possibility, a small sample of tincture of stramonium was concentrated and spotted out directly on the paper. During the run the alkaloids appeared to be separated entirely from other gross coloring matter, and distinct spots

TABLE I.—SUMMARY OF RESULTS OF COMPARATIVE ASSAYS

Preparation	No. of Runs	Densitometric Assay % AH[a]	% S[a]	Total	B. P. Assay, %	Labeled Strength or Official Standard, %
Powdered belladonna extract, U. S. P. XIV	10	0.86	0.23	1.09	1.16	1.25
Powdered belladonna extract with 0.5% hyoscine hydrobromide added	10	0.89	0.69	1.58	1.48	1.75
Powdered stramonium extract, N. F. IX	10	0.17	0.55	0.72	0.80	1.00
Powdered stramonium extract with 0.5% atropine sulfate added	10	0.62	0.64	1.26	1.26	1.50
Belladonna leaf fluidextract, N. F. IX	4	0.22	0.02	0.24	0.34	0.3
Belladonna leaf fluidextract with 0.1% hyoscine hydrobromide added	4	0.27	0.15	0.42	0.43	0.4
Stramonium fluidextract, N. F. IX	4	0.06	0.06	0.12	0.11	0.25
Stramonium fluidextract with 0.1% atropine sulfate added	4	0.11	0.07	0.18	0.19	0.35
Large stramonium leaves from drug garden	4	0.05	0.13	0.18	0.19	0.25
Small stramonium leaves from drug garden	4	0.04	0.16	0.20	0.21	0.25
Young hyoscyamus leaves from drug garden	4	0.019	0.027	0.046	0.039	0.05
Mature hyoscyamus leaves from drug garden	4	0.025	0.030	0.055	0.049	0.05

[a] AH, atropine and hyoscyamine; S, scopolamine.

representing the separated alkaloids were obtained on treatment with the modified Dragendorff's reagent. No quantitative determinations have been made using this method.

It is interesting to note also that relative proportions of atropine-hyoscyamine and scopolamine content are not always in agreement with those found by other workers (5, 6). The variation was most prominent with samples of stramonium, in which the scopolamine formed the largest proportion of the total content.

In runs with stramonium, a third spot having an R_f value of about 0.25 appeared after treatment with modified Dragendorff's reagent. The presence of another alkaloid in stramonium has previously been noted (7, 12).

SUMMARY

1. The application of densitometry to the analysis of the component alkaloids in samples of belladonna, stramonium, and hyoscyamus has been studied. Results of a series of comparative studies have been reported which indicate that the assay procedure can give results which are sufficiently accurate for many purposes. Further work toward simplified extraction procedures for use with this method is advocated.

2. The proportion of the component alkaloids was found to be fairly uniform for samples of products from a particular plant species; however, considerable variation was noted between plant species and between results found by different workers. The proposed method of assay merits further study in the field of analysis of the solanaceous alkaloids.

3. Some evidence to corroborate the presence of a third major component alkaloid in stramonium was noted.

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Some Thyroxine Analogs I.*

Substituted *beta*-Phenylserines and Related Substances

By ROBERT FRIEDENBERG† and W. LEWIS NOBLES‡

The synthesis of a possible thyroxine antagonist is presented. This substituted *beta*-phenylserine was prepared in order to explore the possibility that thyroxine analogs containing a hydroxyl group substituted for a hydrogen atom on the *beta*-carbon atom might prove to be thyroxine antagonists.

AN INSIGHT into the understanding of the action of thyroxine in the body has come about primarily through the synthesis of this hormone (1) and compounds closely related to it. The testing of analogs of this material has become a major source of fundamental knowledge concerning thyromimetic substances and insight into the chemical relationships involved in thyroid disorders.

Since Harington and Barger's original work (1), many analogs of thyroxine have been prepared. The physiological activity of certain analogs, together with the inactivity of others, has led to the postulation of certain structural requirements for thyromimetic action. Selenkow and Asper (2) have recently given an excellent summary of the physiological activity of compounds related to thyroxine and have set forth these "requirements" for thyroxine-like activity.

Unfortunately, study in the field of possible thyroxine antagonists has not advanced to the above point at the present time. Certainly the competitive nature of thyroxine inhibition by structural analogs has not been so clearly delineated as for other antimetabolites. The application of the principle of competitive inhibition of thyroxine analogs with thyroxine at its peripheral site of biochemical activity was announced by Woolley in 1946 (3). This preliminary work has been expanded with the expectation of finding compounds capable of antagonizing the effects of thyroxine either by direct inhibition or by interfering with its production. The present state of our knowledge in this area has also been summarized by Selenkow and Asper (2). They reported that the most active antagonists appeared to fall into one of three major groups: (*a*) derivatives of phenylalanine, (*b*) derivatives of benzoic acid, and (*c*) derivatives of phenoxyacetic acid.

At the present time, however, it is to be noted that no antithyroxine compound thus far studied has inhibited the peripheral effects of endogenously produced thyroxine in normal animals (2). Thus, the field of thyroxine antagonists appears to present a challenge to the thesis propounded by Woolley (4) that "many compounds related structurally in specific ways to metabolically important substances are capable of producing signs of insufficiency of the analogous metabolite."

In other amino acids, compounds prepared by the substitution of a hydrogen atom on the *beta*-carbon with a hydroxyl group had demonstrated competitive antagonism with the metabolite in question. Woolley (5) pointed out that *beta*-hydroxyphenylalanine, *beta*-hydroxyaspartic, and *beta*-hydroxyglutamic acids were antagonists of the related metabolites. This observation is not limited to the simple amino acids, however, since Woolley (6) also pointed out that 2-amino-4,7-dihydroxypteridine-6-carboxylal-*p*-aminobenzoylglutamic acid is a potent antagonist of folic acid. This compound differs from the metabolite in that a hydroxyl group replaces a hydrogen atom at position 7 and an oxygen replaces two hydrogens at position 9. In addition, *p*-aminosalicylic acid, derived from PABA by the exchange of hydrogen for a phenolic hydroxyl group, is an antimetabolite of *p*-aminobenzoic acid.

DISCUSSION

Thus, compounds of the type represented by the following structure(s) were desired to test this hypothesis in the field of thyroxine antagonists:

* Received December 20, 1956, from the School of Pharmacy, University of Mississippi, University.

This investigation was supported in part by research grant No. G-3995 and (C) from the Division of Research Grants of the National Institute of Health, Public Health Service.

Abstracted in part from a dissertation submitted by Robert Friedenberg in partial fulfillment of the requirements for the M.S. degree from the University of Mississippi, 1956.

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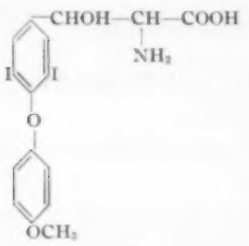
The authors would like to express their appreciation for the kind assistance rendered by Dr. S. B. Barker, Pharmacology Department, University of Alabama Medical Center, and the member of his staff in this work.



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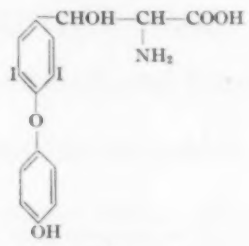

II

The experimental procedures utilized in this work were patterned after those employed by Harington and Barger (1) and Niemann and his associates (7). The synthetic scheme employed by these workers was followed through the synthesis of 3,5-diiodo-4-(4′-methoxyphenoxyaniline). At this point the same difficulty was encountered that had delayed the work of the above-mentioned workers: namely, the preparation and purification of the nitrile obtained from the related aniline derivative. This particular step proceeded, in large runs, with no little difficulty. Ultimately, a procedure was utilized which bypassed the isolation of the nitrile (see experimental section).

EXPERIMENTAL[1]

The methods employed for the synthesis of compounds up to and including 3,5-diiodo-4-(4′-methoxyphenoxy)aniline were those of Harington and Barger (1) and Niemann and Redemann (7).

3,5-Diiodo-4-(4′-methoxyphenoxy)benzonitrile.—Twenty grams of 3,5-diiodo-4-(4′-methoxyphenoxy)-aniline was dissolved in 200 ml. of warm glacial acetic acid. The solution was cooled rapidly to 15° while adding simultaneously 25 ml. of glacial acetic acid which contained 1.5 Gm. anhydrous hydrogen chloride. The solution was diazotized in the following manner: while maintaining a temperature below 20°, 7 ml. of freshly distilled butyl nitrite was added slowly. The resulting mixture was allowed to stand 30 minutes in the cold. The solution of the diazonium salt was then added slowly to a potassium cuprocyanide solution while maintaining the temperature below 10° with vigorous stirring. The potassium cuprocyanide solution was prepared by mixing 114 Gm. potassium cyanide and 100 Gm. of cupric sulfate pentahydrate in 700 ml. water. After the addition of the diazonium solution, the reaction mixture was stirred for one hour at room temperature. The mixture was then warmed momentarily to 80° and then rapidly cooled to 20°, and the solid collected. The crude nitrile was dried for 48 hours at room temperature. In our hands, neither of the reported methods (1, 7) yielded the pure nitrile consistently. On several occasions, the product decomposed resulting in loss of virtually all the material. Noting that the nitrile, in chloroform solution, was to be used in the next step in which the nitrile was to be converted to the aldehyde, an attempt was made to bypass the purification of the nitrile and use the crude product from the above reaction without further purification. Subsequently, it was found that extraction of the crude nitrile with chloroform followed by the addition of a fourfold quantity of ligroin and subsequent concentration in volume would yield a rather pure form of the nitrile. This could then be recrystallized from 2-butanone yielding 8.5 Gm. (42%), m. p. 165–166°. [Literature reports 167–169° (1).]

3,5-Diiodo-4-(4′-methoxyphenoxy)-benzaldehyde.—The following procedure was developed as the most convenient for proceeding from the crude nitrile to the benzaldehyde: five grams of the crude nitrile was placed in a Soxhlet extractor with 50 ml. of chloroform. After extracting for two hours, the dark wine-colored solution was allowed to cool. Petroleum ether (three to fourfold) was added to this solution until no further precipitation occurred. The solution was filtered, the precipitate discarded, and the solution evaporated on a steam bath. At this point the pure nitrile could be isolated by further concentration or, if desired, transferred directly to the chloroform solution without isolation. When the isolation was bypassed, the petroleum ether solution was evaporated almost to dryness and then brought up to 70 ml. volume with chloroform. A proportional quantity (roughly 10 Gm. of stannous chloride in 55 ml. anhydrous ether) of an ethereal solution of stannous chloride was utilized in the following manner: while bubbling anhydrous hydrogen chloride through this ethereal solution at 0°, the nitrile solution was added while agitating the flask. After standing for 30 minutes, the solution was filtered and the filtrate evaporated to dryness on the steam bath. The cooled, dry salt was then hydrolyzed with 100 ml. of boiling 6 *N* hydrochloric acid. The solution was then cooled and the precipitate removed by filtration. The crude product thus obtained was washed with water and then recrystallized from glacial acetic acid. Thus 2.9 Gm. (75% conversion, 80% purity on the original nitrile), m. p. 119–120°, was obtained. [Literature reports 121° (1).]

Anal.—Calcd. for $C_{14}H_{10}I_2O_3$: C, 35.02; H, 2.10. Found: C, 34.89; H, 2.10.

3,5-Diiodo-4-(4′-methoxyphenoxy)-phenylserine.—The following modification of Erlenmeyer's method (8) was found to yield the best results in this case: seven-tenths Gm. (0.01 mole) of glycine was dissolved in 20 ml. water. Four and eight-tenths Gm. (0.01 mole) of the above benzaldehyde was dissolved in 200 ml. ethanol. Ten milliliters of 0.1 *N* sodium hydroxide solution was added to the glycine solution followed by the slow addition of the warm alcoholic benzaldehyde solution. The mix-

[1] Carbon and hydrogen analyses by Weiler and Strauss, Oxford, England.

ture was stirred constantly during addition. A yellow precipitate formed almost immediately. This mixture was allowed to stand for 30 minutes and then the product was removed by filtration and washed with cold water. Thus, 4.5 Gm. (83%) of the desired product was obtained. The product thus obtained was recrystallized from ethanol, m. p. 165–166°.

Anal.—Calcd. for $C_{16}H_{15}I_2NO_5$: C, 34.62; H, 2.72. Found: C, 34.67; H, 2.37.

3,5 - Diiodo - 4 - (4′ - hydroxyphenoxy) - beta-phenylserine.—One-half gram of 3,5-diiodo-4-(4′-methoxyphenoxy) phenylserine was dissolved in 5 ml. of glacial acetic acid and refluxed for one hour with 5 ml. of hydriodic acid, sp. gr. 1.7. The reaction mixture was then poured into 25 ml. of distilled water and allowed to cool. The resulting solid [0.4 Gm. (80%)] was recrystallized from ethanol to a constant melting point of 160°.

Anal.—Calcd. for $C_{15}H_{13}I_2NO_5$: C, 33.29; H, 2.40. Found: C, 33.41; H, 2.44.

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Book Notices

Accidental Poisoning in Childhood. By EDWARD PRESS. Prepared by The Committee on Accident Prevention, American Academy of Pediatrics, Charles C Thomas, Springfield, Illinois, 1956. xiv + 131 pp. 14 x 22 cm.

This is a very useful reference-guide to the chemical constituents of common household substances, together with recommended treatment, by first aid and by the physician, following their accidental ingestion by children. The preliminary edition of this book has been used in Poison Control Centers and by hospitals and physicians, and it has been revised to incorporate many suggestions. It is divided into 3 sections with different colored pages covering: I. treatment procedures; II. list of toxic substances and suggested treatment; III. toxic constituents of household products. The book should be in the hands of all pharmacists and physicians, particularly for its listing of the toxic ingredients of many products that do not give this important information on the label.

Year Book of Drug Therapy (1956–1957 Series). Edited by HARRY BECKMAN. The Year Book Publishers, Inc., Chicago, 1957. 514 pp. 13 x 20 cm. Price $6.75.

This issue of the Year Book continues to reflect Dr. Beckman's excellent coverage of important developments in the field of drug therapy by means of concise, yet thoroughly informative abstracts of reports that were published in journals received between August, 1955 and August, 1956. The editor's usually interesting commentary in the Introduction deals with the current views regarding serotonin (5-hydroxytryptamine) and lysergic acid diethylamide (LSD), the two compounds that have been studied extensively in attempts to elucidate some biochemical relationship for the action of tranquilizing drugs. He concludes the picture is still nebulous. The book is a must for the ready reference library, and its use is facilitated with a good subject index and an author index.

Technique of Organic Chemistry. Vol. III. Part II: Laboratory Engineering. Edited by ARNOLD WEISSBERGER. Interscience Publishers, Inc., New York, 1957. ix + 391 pp. 16 x 23 cm. Price $8.

This second part of volume III includes discussions of the following subjects: selection of materials for the construction of equipment, heating and cooling, grinding, screening, classifying, mixing, and operations with gases. This more selective and specialized part complements the first part of volume III which was reviewed in THIS JOURNAL, **46**, 329(1957). Information that is useful in laboratory development and utilization is included. The thorough documentation extends to the items included in a tabulation of liquids used for heating baths. Appended are an index and a cumulative index for volumes I through IX giving the authors and their subjects and a listing by subjects.

Pharmacists, particularly those in manfacturing, will find the chapters on grinding, screening, classifying, and mixing of practical interest.

Pharmacognosy Laboratory Manual. By MAYNARD W. QUIMBY, RAYMOND W. VANDER WYK, and WILLIAM E. HASSAN, JR. Massachusetts College of Pharmacy, Boston, 1957. viii + 110 pp. 22 x 22.5 cm. Price $3.

The manual is planned to acquaint the student with the techniques required for the study of plants, and divides the laboratory material into parts dealing with harmful plants and drugs and products of economic importance of biological origin. Books

and manuals being prepared for pharmacy students, and particularly those dealing with official drugs, should not change the style of the official monographs by reverting to the use of cc. for ml., mgms. for mg., or laevo- for levo-. The questions posed for the students should produce useful results.

Rauwolfia: Botany, Pharmacognosy, Chemistry and Pharmacology. By ROBERT E. WOODSON, JR., HEBER W. YOUNGKEN, EMIL SCHLITTLER, and JURG A. SCHNEIDER. Little, Brown and Company, Boston, 1957. xii + 149 pp. 16 x 24 cm. Price $5.50.

This is a useful compilation of material on one of the plant drugs that have renewed interest in plant sources of drugs and drug intermediates for synthesis. The text is divided into four parts, each part the work of a specialist, as follows: The Botany of Rauwolfia by R. E. Woodson, Jr., The Pharmacognosy of Rauwolfia by H.W. Youngken, The Chemistry of Rauwolfia Alkaloids by E. Schlittler, The Pharmacology of Rauwolfia by J. A. Schneider. The chapter on Chemistry is particularly interesting and complete. It indicates how rapidly investigators move from the stage of separation to identification and structural elucidation, and finally to synthesis of naturally occurring compounds. The text is illustrated, many clear structural formulas are included, and each chapter is documented with references. An index is appended. The book is an excellent monograph and should be helpful and interesting to pharmaceutical chemists, and to teachers and students in pharmacy and related fields.

Heterocyclic Compounds. Vol. 5. Edited by ROBERT C. ELDERFIELD. John Wiley & Sons, Inc., New York, 1957. vi + 744 pp. 15.5 x 23.5 cm. Price $20.

This fifth volume of the series on heterocyclic compounds covers five-membered heterocycles containing two hetero atoms and their benzo derivatives. The editor notes the omission of the 1,2-dioxolanes and the inclusion of the 1,3-dioxolanes. The 1,2-dioxolanes are held to resemble conventional peroxides so closely in their behavior that separate treatment is unnecessary. The value of *Chemical Abstracts* in the preparation of a comprehensive chemical treatise is indicated in the explanation that one of the factors that caused serious editorial problems was the death within the past two years of one of the senior editors of *Chemical Abstracts*, resulting in delay between the appearance of a paper (presumably foreign) and the appearance of an abstract thereof. It has been four years since volumes 3 and 4 appeared and were reviewed in THIS JOURNAL, **41**, 516(1952).

The style of including the numerous references to original literature on current pages is continued, as is the good format, selection of type, and clear structural formulas. A more extensive index is appended to this volume than to the earlier volumes. This book will be a useful addition to all chemical libraries, and it is hoped that volume 6, which will cover six-membered heterocycles with two hetero atoms and their benzo derivatives, will not be delayed in its publication.

Encyclopedia of Chemical Technology. Vol. XV. (Waxes to Zymosterol and Index to Volumes 1–15.) Edited by RAYMOND E. KIRK and DONALD F. OTHMER. Interscience Publishers, Inc., New York, 1956. xiv + 936 pp. 19.5 x 27 cm. Price $30 (Subscription Price $25).

This is the last volume of a valuable compilation of useful and documented information for chemists and workers in related fields. The main flaw, which is unavoidable in any publication that requires a span of years for presentation, is the rapidity with which new material accumulates and cannot be included in texts already printed or in the process of publication. The editors recognize this and indicate that a supplement volume devoted to subjects in which recent advances have been particularly striking, will be published in the fall of 1957, and that other supplement volumes will be issued when necessary.

The fifteenth volume of this handsomely printed and bound series contains only 313 pages of text covering the alphabetically arranged material from Waxes through Zymosterol. It also contains 620 pages devoted to the cumulative index for the entire series of 15 volumes. The value of this encyclopedia to anyone requiring ready access to a comprehensive source of reliable information is obvious. All technical and general libraries should make this important work available to its readers.

Enzyme Antigen and Virus. By F. MACFARLANE BURNET. Cambridge University Press, New York, 1956. viii + 193 pp. 12.5 x 19 cm. Price $3.50.

This book attempts to relate protein synthesis to the production of adaptive enzymes, stimulation of antibody production by antigen, and the intracellular multiplication of influenza and other viruses. An attempt is made to sketch an "information theory" of the cell in which macromolecular pattern serves as a means of conveying "information" within the organism. The text is divided under the following headings: enzyme action and protein synthesis, antibody production, the self-marker hypothesis in relation to cellular proliferation and control, virus multiplication, and the scope of biological generalization.

Notes on Atomic Energy for Medical Officers. By the Staff of the Royal Naval Medical School, Alverstoke, Hampshire, England. Philosophical Library, Inc., New York, 1956. v + 169 pp. 12 x 18 cm. Price $4.75.

This pocket-size book provides in concise and simplified form basic information for military physicians on radiations (particularly those resulting from nuclear explosions and other kinds of radiological warfare); the short and long term effects, the dangers, methods of protection against radiations, and the treatment of radiation casualties. Brief appendixes explain the source of atomic energy, the permissible hazards for industrial purposes, uses of isotopes, and the hydrogen bomb. A glossary and an index are included.

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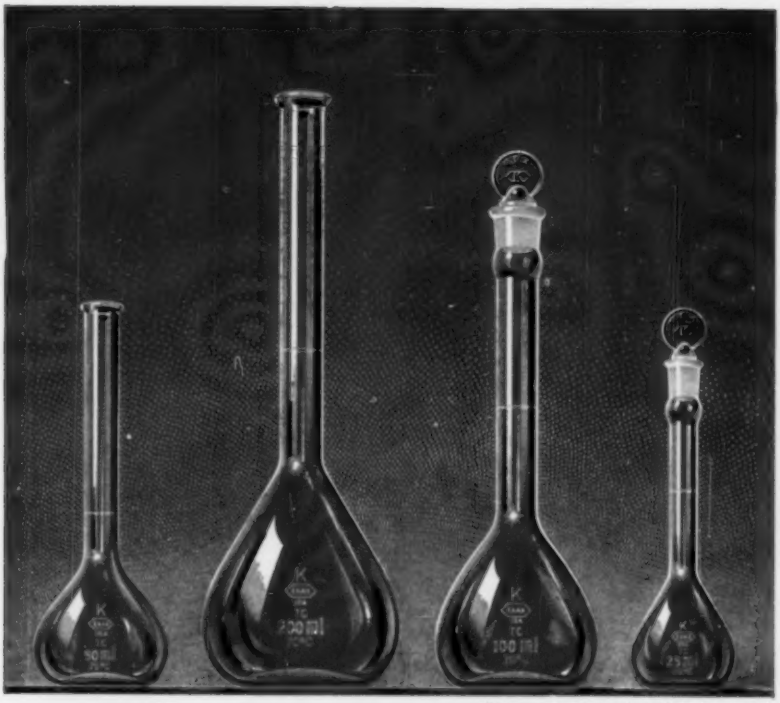

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